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(54) **Imidazolinone resistant ahas mutants.**

(57) The present invention relates to monocot genes encoding a mutant AHAS enzyme that is specifically resistant to imidazolinone herbicides. Exemplary of these genes are corn DNA sequences which encode an amino acid substitution at position 621 of the wild-type AHAS enzyme. The mutant gene can be used to transform other plants to herbicide resistance; in this regard, the invention also provides host cells and vectors containing the gene, which cells and vectors are useful in the transformation process.

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This invention relates to novel DNA sequences that encode novel variant forms of acetohydroxy acid synthase enzyme (hereinafter AHAS). The AHAS enzyme is a critical enzyme routinely produced in a variety of plants and a broad range of microorganisms. Normal AHAS function is inhibited by imidazolinone herbicides; however, new AHAS enzymes encoded by the mutant DNA sequences function normally catalytically even in the presence of imidazolinone herbicides and, therefore, confer herbicide resistance upon the plant or microorganism containing them.

The novel DNA sequences are derived from corn and have a substitution of an amino acid at position 621 of the normal AHAS sequence. This substitution in the AHAS gene sequence results in a fully functional enzyme, but renders the enzyme specifically resistant to inhibition by a variety of imidazolinone herbicides. The availability of these variant sequences provides a tool for transformation of different crop plants to imidazolinone herbicide resistance, as well as providing novel selectable markers for use in other types of genetic transformation experiments.

BACKGROUND OF THE INVENTION

The use of herbicides in agriculture is now widespread. Although there are a large number of available compounds which effectively destroy weeds, not all herbicides are capable of selectively targeting the undesirable plants over crop plants as well as being non-toxic to animals. Often, it is necessary to settle for compounds which are simply less toxic to crop plants than to weeds. In order to overcome this problem, development of herbicide resistant crop plants has become a major focus of agricultural research.

An important aspect of development of herbicide-resistance is an understanding of the herbicide target, and then manipulating the affected biochemical pathway in the crop plant so that the inhibitory effect is avoided while the plant retains normal biological function. One of the first discoveries of the biochemical mechanism of herbicides related to a series of structurally unrelated herbicide compounds, the imidazolinones, the sulfonylureas and the triazopyrimidines. It is now known (Shaner et al. *Plant Physiol.* 76: 545-546, 1984; U.S. Patent No. 4,761,373) that each of these herbicides inhibits plant growth by interference with an essential enzyme required for plant growth, acetohydroxyacid synthase (AHAS; also referred to as acetolactate synthase, or ALS). AHAS is required for the synthesis of the amino acids isoleucine, leucine and valine.

The AHAS enzyme is known to be present throughout higher plants, as well as being found in a variety of microorganisms, such as the yeast *Saccharomyces cerevisiae*, and the enteric bacteria, *Escherichia coli* and *Salmonella typhimurium*. The genetic basis for the production of normal AHAS in a number of these species has also been well characterized. For example, in both *E. coli* and *S. typhimurium* three isozymes of AHAS exist; two of these are sensitive to herbicides while a third is not. Each of these isozymes possesses one large and one small protein subunit; and map to the *llvH*, *llvGM* and *llvBN* operons. In yeast, the single AHAS isozyme has been mapped to the *ILV2* locus. In each case, sensitive and resistant forms have been identified and sequences of the various alleles have been determined (Friden et al., *Nucl. Acid Res.* 13: 3979-3993, 1985; Lawther et al., *PNAS USA* 78: 922-928, 1982; Squires et al., *Nucl. Acids Res* 11: 5299-5313, 1983; Wek et al, *Nucl. Acids Res* 13: 4011-4027, 1985; Falco and Dumas, *Genetics* 109, 21-35, 1985; Falco et al, *Nucl. Acids Res* 13: 4011-4027, 1985).

In tobacco, AHAS function is encoded by two unlinked genes, *SuRA* and *SuRB*. There is substantial identity between the two genes, both at the nucleotide level and amino acid level in the mature protein, although the N-terminal, putative transit region differs more substantially (Lee et al, *EMBO J.* 7: 1241-1248, 1988). *Arabidopsis*, on the other hand, has a single AHAS gene, which has also been completely sequenced (Mazur et al., *Plant Physiol.* 85:1110-1117, 1987). Comparisons among sequences of the AHAS genes in higher plants indicates a high level of conservation of certain regions of the sequence; specifically, there are at least 10 regions of sequence conservation. It has previously been assumed that these conserved regions are critical to the function of the enzyme, and that retention of that function is dependent upon substantial sequence conservation.

It has been recently reported (U.S. Patent No. 5,013,659) that mutants exhibiting herbicide resistance possess mutations in at least one amino acid in one or more of these conserved regions. In particular, substitution of certain amino acids for the wild type amino acid at these specific sites in the AHAS protein sequence have been shown to be tolerated, and indeed result in herbicide resistance of the plant possessing this mutation, while retaining catalytic function. The mutations described therein encode either cross resistance for imidazolinones and sulfonylureas or sulfonylurea-specific resistance, but no imidazolinone-specific mutations were disclosed. These mutations have been shown to occur at both the *SuRA* and *SuRB* loci in tobacco; similar mutations have been isolated in *Arabidopsis* and yeast.

Imidazolinone-specific resistance has been reported elsewhere in a number of plants. U.S. Patent No.

4,761,373 generally described an altered AHAS as a basis of herbicide resistance in plants, and specifically disclosed certain imidazolinone resistant corn lines. Haughn et al. (Mol. Gen. Genet. 211:266-271, 1988) disclosed the occurrence of a similar phenotype in *Arabidopsis*. Sathasivan et al. (Nucl. Acid Res. 18:2188, 1990) identified the imidazolinone-specific resistance in *Arabidopsis* as being based on a mutation at position 653 in the normal AHAS sequence. In accordance with the present invention, a gene encoding imidazolinone-specific resistance in a monocot has now been isolated and determined to be associated with a single amino acid substitution in a wild-type monocot AHAS amino acid sequence.

SUMMARY OF THE INVENTION

The present invention provides novel nucleic acid sequences encoding functional monocot AHAS enzymes insensitive to imidazolinone herbicides. The sequences in question comprise a mutation in the codon encoding the amino acid serine at position 621 in the corn (maize) AHAS sequence, or in the corresponding position in other monocot sequences. Other monocots, such as wheat, are also known to exhibit imidazolinone specific mutations (e.g., ATCC Nos. 40994-97). In corn, the wild type sequence has a serine at this position. In a preferred embodiment, the substitution is asparagine for serine, but alternate substitutions for serine include aspartic acid, glutamic acid, glutamine and tryptophane. Although the claimed sequences are originally derived from monocots, the novel sequences are useful in methods for producing imidazolinone resistant cells in any type of plant, said methods comprising transforming a target plant cell with one or more of the altered sequences provided herein. Alternatively, mutagenesis is utilized to create mutants in plant cells or seeds containing a nucleic acid sequence encoding an imidazolinone insensitive AHAS. In the case of mutant plant cells isolated in tissue culture, plants which possess the imidazolinone resistant or insensitive trait are then regenerated. The invention thus also encompasses plant cells, bacterial cells, fungal cells, plant tissue cultures, adult plants, and plant seeds that possess a mutant nucleic acid sequence and which express functional imidazolinone resistant AHAS enzymes.

The availability of these novel herbicide resistant plants enables new methods of growing crop plants in the presence of imidazolinones. Instead of growing non-resistant plants, fields may be planted with the resistant plants produced by mutation or by transformation with the mutant sequences of the present invention, and the field routinely treated with imidazolinones, with no resulting damage to crop plants.

The mutant nucleic acids of the present invention also provide novel selectable markers for use in transformation experiments. The nucleic acid sequence encoding a resistant AHAS is linked to a second gene prior to transfer to a host cell, and the entire construct transformed into the host. Putative transformed cells are then grown in culture in the presence of inhibitory amounts of herbicide; surviving cells will have a high probability of having successfully acquired the second gene of interest. Alternately, the resistant AHAS gene can be cotransformed on an independent plasmid with the gene of interest, whereby about 50% of all transformants can be expected to have received both genes.

The following definitions should be understood to apply throughout the specification and claims. A "functional" or "normal" AHAS enzyme is one which is capable of catalyzing the first step in the pathway for synthesis of the essential amino acids isoleucine, leucine and valine. A "wild-type" AHAS sequence is a sequence present in an imidazolinone sensitive member of a given species. A "resistant" plant is one which produces a mutant but functional AHAS enzyme, and which is capable of reaching maturity when grown in the presence of normally inhibitory levels of imidazolinone. The term "resistant", as used herein, is also intended to encompass "tolerant" plants, i.e., those plants which phenotypically evidence adverse, but not lethal, reactions to the imidazolinone.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: AHAS enzyme activity in 10-day old maize seedlings (corn lines A619 or X112) in the presence of imazethapyr (Pursuit™ A) or chlorsulfuron (Oust™ B). Herbicide resistant AHAS activity is calculated as percentage of AHAS activity in the absence of inhibitor. The standard error between experiments is 10%.

Figure 2: Southern analysis of genomic clones in phage EMBL3. Phages 12-1A (from W22), 12-7A, 18-8A, 12-11, and 12-17A (From X112) are digested with XbaI or Sall, separated on a 1% agarose gel, transferred onto nitrocellulose and hybridized with an AHAS cDNA fragment as probe.

Figure 3: Southern analysis of genomic DNA from corn lines X112, XA17, QJ22, A188 and B73. The DNA is digested with XbaI, separated on a 1% agarose gel, transferred onto nitrocellulose and hybridized with an AHAS cDNA fragment as probe.

Figure 4: Restriction map of plasmid pCD8A. The mutant AHAS gene from X112 was subcloned as a

8kb PstI fragment into vector pKS(+). The location and orientation of the AHAS gene is indicated by an arrow. The restriction sites of PstI, XhoI, HindIII, XbaI and ClaI are represented by symbols.

Figure 5: Nucleotide sequencing gel of the non-coding strand (A) and the double stranded DNA sequence (B) of AHAS clones W22/4-4, B73/10-4 and XI12/8A in the region of amino acids 614 to 633. The position of the cytosine to thymidine transition is indicated by an arrow.

Figure 6: Nucleotide and deduced amino acid sequences of the XI12/8A mutant AHAS gene.

Figure 7: Nucleotide sequence alignment of XI12/8A, B73/7-4 and W22/1A *als2* genes. (*) marks the base change causing the mutation at position 621, (#) differences from the B73/7-4 sequence and (>) represents silent changes.

Figure 8: Amino acid sequences and alignment of XI12/8A, B73/7-4 and W22/1A *als2* genes. (*) marks the mutation at position 621, (#) marks differences from the B73/7-4 sequence, and (>) represents silent changes.

DETAILED DESCRIPTION OF THE INVENTION

The gene of the present invention is isolatable from corn maize line XI12 (seed deposited with the American Type Culture Collection as Accession Number 75051), and has been inserted into plasmid pXI12/8A (deposited with the American Type Culture Collection as Accession Number 68643). It is also isolatable from any other imidazolinone-specific herbicide resistant mutant, such as the corn line QJ22 (deposited as a cell culture with the American Type Culture Collection as Accession Number 40129), or the various wheat plants (seed deposited with the American Type Collection as Accession Numbers 40994, 40995, 40996, or 40997). A genomic DNA library is created, for example, in phage ENBL-3 with DNA from one of the imidazolinone resistant mutants, preferably one which is homozygous for the resistance trait, and is screened with a nucleic acid probe comprising all or a part of a wild-type AHAS sequence.

In maize, the AHAS gene is found at two loci, *als1* and *als2* (Burr and Burr, Trends in Genetics 7:55-61, 1991); the homology between the two loci is 95% at the nucleotide level. The mutation in XI12 is mapped to locus *als2* on chromosome 5, whereas the nonmutant gene is mapped to locus *als1* on chromosome 4 (Newhouse et al., "Imidazolinone-resistant crops". In *The Imidazolinone Herbicides*, Shaner and O'Connor. (Eds.), CRC Press, Boca Raton, FL, in Press). Southern analysis identifies some clones containing the mutant *als2* gene, and some containing the non-mutant *als1* gene. Both types are subcloned into sequencing vectors, and sequenced by the dideoxy sequencing method.

Sequencing and comparison of wild type and mutant AHAS genes shows a difference of a single nucleotide in the codon encoding the amino acid at position 621 (Figure 5). Specifically, the codon AGT encoding serine in the wild type is changed to AAT encoding asparagine in the mutant AHAS (Figure 8). The mutant AHAS gene is otherwise similar to the wild type gene, encoding a protein having 638 amino acids, the first 40 of which constitute a transit peptide which is thought to be cleaved during transport into the chloroplast *in vivo*. The sequence of the *als1* non-mutant gene from XI12 appears to be identical to the *als1* gene from B73.

The mutant genes of the present invention confer resistance to imidazolinone herbicides, but not to sulfonyleurea herbicides. Types of herbicides to which resistance is conferred are described for example in U.S. Patent Nos. 4,188,487; 4,201,565; 4,221,586; 4,297,128; 4,554,013; 4,608,079; 4,638,068; 4,747,301; 4,650,514; 4,698,092; 4,701,208; 4,709,036; 4,752,323; 4,772,311; and 4,798,619.

It will be understood by those skilled in the art that the nucleic acid sequence depicted in Figure 6 is not the only sequence which can be used to confer imidazolinone-specific resistance. Also contemplated are those nucleic acid sequences which encode an identical protein but which, because of the degeneracy of the genetic code, possess a different nucleotide sequence. The invention also encompasses genes encoding AHAS sequences in which the aforementioned mutation is present, but which also encode one or more silent amino acid changes in portions of the molecule not involved with resistance or catalytic function. Also contemplated are gene sequences from other imidazolinone resistant monocots which have a mutation in the corresponding region of the sequences.

For example, alterations in the gene sequence which result in the production of a chemically equivalent amino acid at a given site are contemplated; thus, a codon for the amino acid alanine, a hydrophobic amino acid, can readily be substituted by a codon encoding another hydrophobic residue, such as glycine, or may be substituted with a more hydrophobic residue such as valine, leucine, or isoleucine. Similarly, changes which result in substitution of one negatively charged residue for another, such as aspartic acid for glutamic acid, or one positively charged residue for another, such as lysine for arginine, can also be expected to produce a biologically equivalent product. The invention also encompasses chimaeric genes, in which the substituted portion of the corn AHAS gene is recombined with unaltered portions of the normal AHAS genes

from other species. Thus, throughout the specification and claims, wherever the term "imidazolinone-specific resistant corn AHAS gene" is used, it is intended to cover each of these alternate embodiments as well as the sequence of Figure 6.

Isolated AHAS DNA sequences of the present invention are useful to transform target crop plants, and thereby confer imidazolinone resistance. A broad range of techniques currently exist for achieving direct or indirect transformation of higher plants with exogenous DNA, and any method by which the novel sequence can be incorporated into the host genome, and stably inherited by its progeny, is contemplated by the present invention. The imidazolinone specific resistance trait is inherited as a single dominant nuclear gene. The level of imidazolinone resistance is increased when the gene is present in a homozygous state; such corn plants, for example, have a resistance level of about 1,000 times that of a non-resistant plant. Plants heterozygous for the trait, however, have a resistance of about 50-500 times that of a non-resistant plant.

Transformation of plant cells can be mediated by the use of vectors. A common method of achieving transformation is the use of *Agrobacterium tumefaciens* to introduce a foreign gene into the target plant cell. For example, the mutant AHAS sequence is inserted into a plasmid vector containing the flanking sequences in the Ti-plasmid T-DNA. The plasmid is then transformed into *E. coli*. A triparental mating among this strain, an *Agrobacterium* strain containing a disarmed Ti-plasmid containing the virulence functions needed to effect transfer of the AHAS containing T-DNA sequences into the target plant chromosome, and a second *E. coli* strain containing a plasmid having sequences necessary to mobilize transfer of the AHAS construct from *E. coli* to *Agrobacterium* is carried out. A recombinant *Agrobacterium* strain, containing the necessary sequences for plant transformation is used to infect leaf discs. Discs are grown on selection media and successfully transformed regenerants are identified. The recovered plants are resistant to the effects of herbicide when grown in its presence. Plant viruses also provide a possible means for transfer of exogenous DNA.

Direct uptake of plant cells can also be employed. Typically, protoplasts of the target plant are placed in culture in the presence of the DNA to be transferred, and an agent which promotes the uptake of DNA by protoplast. Useful agents in this regard are polyethylene glycol or calcium phosphate.

Alternatively, DNA uptake can be stimulated by electroporation. In this method, an electrical pulse is used to open temporary pores in a protoplast cell membrane, and DNA in the surrounding solution is then drawn into the cell through the pores. Similarly, microinjection can be employed to deliver the DNA directly into a cell, and preferably directly into the nucleus of the cell.

In each of the foregoing techniques, transformation occurs in a plant cell in culture. Subsequent to the transformation event, plant cells must be regenerated to whole plants. Techniques for the regeneration of mature plants from callus or protoplast culture are now well known for a large number of different species (see, e.g., *Handbook of Plant Cell Culture*, Vols. 1-5, 1983-1989 McMillan, N.Y.) Thus, once transformation has been achieved, it is within the knowledge in the art to regenerate mature plants from the transformed plant cells.

Alternate methods are also now available which do not necessarily require the use of isolated cells, and therefore, plant regeneration techniques, to achieve transformation. These are generally referred to as "ballistic" or "particle acceleration" methods, in which DNA coated metal particles are propelled into plant cells by either a gunpowder charge (Klein et al., *Nature* 327: 70-73, 1987) or electrical discharge (EPO 270 356). In this manner, plant cells in culture or plant reproductive organs or cells, e.g. pollen, can be stably transformed with the DNA sequence of interest.

In certain dicots and monocots direct uptake of DNA is the preferred method of transformation. For example, in corn, the cell wall of cultured cells is digested in a buffer with one or more cell wall degrading enzymes, such as cellulase, hemicellulase and pectinase, to isolate viable protoplasts. The protoplasts are washed several times to remove the enzymes, and mixed with a plasmid vector containing the gene of interest. The cells can be transformed with either PEG (e.g. 20% PEG 4000) or by electroporation. The protoplasts are placed on a nitrocellulose filter and cultured on a medium with embedded corn cells functioning as feeder cultures. After 2-4 weeks, the cultures in the nitrocellulose filter are placed on a medium containing about 0.32 μ M of the imidazolinone and maintained in the medium for 1-2 months. The nitrocellulose filters with the plant cells are transferred to fresh medium with herbicides and nurse cells every two weeks. The untransformed cells cease growing and die after a few weeks.

The present invention can be applied to transformation of virtually any type of plant, both monocot and dicot. Among the crop plants for which transformation to herbicide resistance is contemplated are corn, wheat, rice, millet, oat, barley, sorghum, sunflower, sweet potato, alfalfa, sugar beet, *Brassica* species, tomato, pepper, soybean, tobacco, melon, squash, potato, peanut, pea, cotton, or cacao. The novel sequences may also be used to transform ornamental species, such as rose, and woody species, such as pine and poplar.

The novel sequences of the invention also are useful as selectable markers in plant genetics studies. For example, in plant transformation, sequences encoding imidazolinone resistance could be linked to a gene of interest which is to be used to transform a target imidazolinone sensitive plant cell. The construct comprising both the gene of interest and the imidazolinone resistant sequence are introduced into the plant cell, and the plant cells are then grown in the presence of an inhibitory amount of an imidazolinone. Alternately, the second gene of interest can be cotransformed, on a separate plasmid, into the host cells. Plant cells surviving such treatment presumably have acquired the resistance gene as well as the gene of interest, and therefore, only transformants survive the selection process with the herbicide. Confirmation of successful transformation and expression of both genes can be achieved by Southern hybridization of genomic DNA, by PCR or by observation of the phenotypic expression of the genes.

The invention is further illustrated by the following non-limiting examples.

EXAMPLES

1. Confirmation of Whole Plant Herbicide Resistance in XI12

XI12 plants are treated with herbicides at 10 days to the V3 leaf stage (4-5 leaves, of which 3 have visible ligules). Imazethapyr is applied at rates of 2000, 500, 250, 125 and 62.5 g/ha. Chlorsulfuron is applied at 32, 16, 8, 4 and 2 g/ha. Plants are treated postemergence at a spray volume of 400 l/ha. After spraying, plants are placed in the greenhouse for further observation.

XI12 plants are unaffected at all rates of imazethapyr treatment; however, no visible increased resistance to chlorsulfuron is noted. Thus, XI12 displays selective resistance to the imidazolinone at the whole plant level (See Figure 1).

The resistance in XI12 is also shown to be inherited as a single dominant allele of a nuclear gene. Heterozygous resistant XI12 are selfed, and the selfed progeny segregate in the 3 resistant:1 susceptible ratio expected for a single dominant allele of a nuclear gene. In this study, the segregating seedlings are sprayed postemergence with lethal doses of imazethapyr (125 or 250 g/ha) following spraying protocols described above, to establish segregation for resistance.

2. AHAS Extraction

Seeds of XI12 are sown in soil in a greenhouse maintained at day/night temperature of 80°C and 15 hour photoperiod. Plants are harvested two weeks after planting. The basal portion of the shoot is used for the extraction of AHAS. 5 g of the tissue is powdered in liquid nitrogen and then homogenized in AHAS assay buffer comprising 100 mM potassium phosphate buffer (pH 7.5) containing 10 mM pyruvate, 5 mM MgCl₂, 5 mM EDTA, 100 µM FAD (flavin adenine dinucleotide), 1 mM valine, 1 mM leucine, 10% glycerol and 10 mM cysteine. The homogenate is centrifuged at 10,000 rpm for 10 minutes and 3 ml of the supernatant are applied onto an equilibrated Bio-Rad Econo-Desalting column (10 DG) and eluted with 4 ml AHAS assay buffer.

AHAS activity is measured by estimation of the product, acetolactate, after conversion by decarboxylation in the presence of acid to acetoin. Standard reaction mixtures contain the enzyme in 50 mM potassium phosphate (pH 7.0) containing 100 mM sodium pyruvate, 10 mM MgCl₂, 1 mM thiamine pyrophosphate, 10 µM FAD, and appropriate concentrations of different inhibitors. This mixture is incubated at 37°C for 1 to 3 hours depending upon the experiment. At the end of this incubation period, the reaction is stopped with the addition of H₂SO₄ to make a final concentration of 0.85% H₂SO₄ in the tube. The reaction product is allowed to decarboxylate at 60°C for 15 minutes. The acetoin formed is determined by incubating with creatine (0.17%) and 1-naphthol (1.7% in 4N NaOH). The absorption of color complex formed is measured at 520 nm.

AHAS activity from B73, A619, or other wild-type maize lines is highly sensitive to inhibition by imazethapyr (PURSUIT™) with an I₅₀ of 1 µM (See Figure 1). Contrary to this observation, XI12 shows 70-80% of enzyme activity at the highest concentrations (100 µM) of PURSUIT™ or ARSENAL™ (imazapyr), and about 70% in the presence of SCEPTER™ (imazequin). This result shows a 100-fold increase in tolerance of AHAS activity from XI12 to imazethapyr as compared to the in vitro AHAS activity from A619. sensitivity of AHAS activity from the two lines to sulfonylureas gives a different picture. In the presence of OUST™ (sulfometuron methyl), at 100 nM, AHAS activity of XI12 is only 15-20%. AHAS activity of A619 in the presence of OUST™ is 5-10%, and in the presence of PURSUIT™ is 15-20% (See Figure 1).

3. Cloning of XI12 AHAS Genes

Seeds of the XI12 mutant derived from an imidazollnone resistant corn tissue culture line are planted; plants obtained therefrom appear to be segregating for the mutant AHAS phenotype. In order to obtain homozygous resistant seed material, a population of XI12 mutant plants are selfed. After selecting for herbicide resistance for three consecutive growing seasons, the seeds are homozygous for the mutant AHAS gene. Homozygous seeds are collected and used to grow seedlings to be used in AHAS gene isolation.

DNA is extracted from 7 days old etiolated seedlings of a homozygous XI12 line. 60 g of plant tissue is powdered in liquid nitrogen, and transferred into 108 ml DNA extraction buffer (1.4 M NaCl, 2.0% Ctab (hexadecyl trimethyl ammonium bromide), 100 mM Tris-Cl pH 8.0, 20 mM EDTA, 2% Mercaptoethanol) and 54 ml water. After incubation at 50-60°C for 30 minutes the suspension is extracted with an equal amount of chloroform. The DNA is precipitated by adding an equal amount of precipitation buffer (1% Ctab, 50 mM Tris-Cl pH 8.0, 10 mM EDTA). To purify the genomic DNA, a high speed centrifugation in 6.6M CsCl and ethidium bromide is performed (Ti80 rotor, 50,000 rpm, 20°C, 24 hours). The purified DNA is extracted with salt saturated Butanol and dialyzed for 25 hours against 3 changes of 1 l dialysis buffer (10 mM Tris-Cl pH 8.0, 1 mM EDTA, 0.1M NaCl). The concentration of the XI12 genomic DNA is determined spectrophotometrically to be 310 mg/ml. The yield is 0.93 mg.

The XI12 genomic DNA is used to create a genomic library in the phage EMBL-3. The DNA is partially digested with MboI and the fragments are separated on a sucrose gradient to produce size range between 8 to 22 kb before cloning into the BamHI site of EMBL-3. After obtaining 2.1×10^6 independent clones, the library is amplified once. The titer of the library is determined 9×10^{10} pfu/ml.

To obtain probes for analysis of the XI12 library, a W22 (wild-type) cDNA library in lambda gt11, purchased from Clontech Inc., CA, is screened with an 800 nt BamHI probe isolated from Arabidopsis AHAS genomic clone. The phages are plated in a density of 50,000 pfu/15 cm plate, transferred onto nitrocellulose filters, prehybridized in 6x SSC, 0.2% SDS for 2 hours and hybridized with the Arabidopsis AHAS probe in 6x SSC, 0.2% SDS overnight. One positive phage is identified, further purified and used for subcloning of a 1.1 kb EcoRI fragment. The 1.1 kb EcoRI fragment is subcloned into pGemA-4 and used as a probe to identify the XI12 AHAS genes.

The XI12 genomic library is plated on 12 15-cm plates (concentration of 50,000 pfu/plate) and is screened with the W22 AHAS cDNA probe. The filters are prehybridized (2 hours) and hybridized (overnight) in Church buffer (0.5 M Na Phosphate, 1 mM EDTA, 1% BSA, 7% SDS) at 65°C and washed at 85°C in 2x SSC, 0.2% SDS and 0.3 x SSC, 0.2% SDS. 12 positive plaques are obtained from a total of 7.5×10^5 pfu screened and 5 positive clones are further purified and isolated according to Chisholm (BioTechniques 7:21-23, 1989). Southern analysis (See Figure 2) showed that the phage clones represented two types of AHAS clones: Type-1 clones contain one large XbaI (>6.5 kb) fragment hybridizing to the AHAS cDNA probe, Type-2 clones contained two 2.7 and 3.7 kb XbaI fragments hybridizing to the AHAS cDNA probe. Genomic Southern of XI12 DNA demonstrated, that the XbaI fragments obtained by digesting genomic DNA and by hybridizing to the AHAS cDNA probe correspond to the XbaI fragments identified in the XI12 phage clones (See Figure 3). Restriction digest and Southern Analysis also demonstrate that of the 5 AHAS clones, one clone represents the mutant als2 genes and four represent the als1 gene.

The AHAS genes corresponding to the mutant locus located on chromosome 5 (clone 12/8A) and the non-mutant locus located on chromosome 4 (clone 12/17A) are subcloned as a PstI fragment (clone 12/8A) or as XbaI fragment (12/17A) into the sequencing vector pBluescript II KSm13(+) (pKS+; Stratagene). Both 2.7 kb and 3.7 kb XbaI fragments from phage 12/17A contain one complete copy of AHAS genes which are identified. The sequence of each is obtained by dideoxy sequencing (Pharmacia T7 sequencing Kits) using primers complementary to the AHAS coding sequence.

The methods of DNA extraction, cloning of the genomic library and screening of the library are as described for the XI12 genomic DNA. The B73 AHAS genes are subcloned into the sequencing vector pKS+ as XbaI fragments and are sequenced. The sequence is obtained by dideoxy sequencing, using primers complementary to the AHAS coding sequence as described for the SI12 AHAS genes.

A W22 genomic library in EMBL3 purchased from Clontech Inc., CA is screened. The phages are plated in a density of 50,000 pfu/7 inch plate, transferred onto nitrocellulose filters, and hybridized with the W22 AHAS cDNA probe described above (prehybridization and hybridization conditions: 6 x SSC, 0.5% SDS, 1X Denhard's 100 mg/ml calf thymus DNA, 65°C, washing conditions: 3X x SSC, 0.2% SDS for 2 hours at 65°C, and 0.3 x SSC, 0.2% SDS for 2 hours). Two positive phages (12/1A and 12/4-4) are identified and further purified.

The W22 genomic clone 12/1A is subcloned as two 0.78 kb (pGemA-4) and 3.0 kb (pGemA-14; Promega) SalI fragments into the vector pGem-A2, and as a 6.5 kb XbaI fragment into the vector pKS+ (pCD200). The coding strand sequence of the W22 AHAS gene is obtained by dideoxy sequencing of

nested deletions created from subclones pGem A-14 and pGem A-4 of phage 12-1A. This sequence is used to design oligonucleotides complementary to the AHAS non-coding strand. The sequence of the non-coding strand is obtained by dideoxy sequencing of clone pCD200 using primers complementary to the coding strand. Upon complementing the sequencing of the W22 AHAS gene, primers of both DNA strands are designed and used for the sequencing of the AHAS genes isolated from the XI12 and B73 genomic libraries.

4. Cloning of QJ22 AHAS Genes

The sequence of the gene encoding AHAS in the maize line QJ22, which is selectively resistant to imidazolinones, is also determined. A genomic library of QJ22 is prepared in an EMBL3 vector. A library of 800,000 phage is screened with an 850 nucleotide SalI/ClaI fragment isolated from an AHAS clone (A-4) derived from the wild-type maize line W22. Five positive phages are picked and submitted to a second round of screening to partially purify the phage. The partially purified phage are analyzed by PCR to determine if any clones represent the QJ22 als1 gene. Such clones are identified as a 3.7kb XbaI fragment with a gene specific SmaI site at position 495. The second screen indicates the presence of a single positive clone with these characteristics.

The PCR product is purified using a commercial kit (Magic-PCR Preps) from Promega; and the purified DNA is sequenced with a Taq polymerase sequencing system "fmol", also from Promega. Sequence analysis of both strands of the DNA of the QJ22 mutant AHAS shows a nucleotide transition from G to A in the codon for amino acid 621. This mutation is identical to the one seen in XI12 and the remainder of the sequence is typical of an als1 gene.

RESULTS

The sequence of the mutant AHAS genes is compared with the sequences obtained from the wild type corn lines B73 and W22 (See Figure 7). The XI12 mutant gene (XI12/8A) and the QJ22 mutant gene and the wild type gene are identical except for the amino acid change at position 621, causing a single nucleotide transition from AGT to AAT (See Figure 8). The XI12 mutant XI12/8A and the wild-type B73/7-4 gene show an additional difference at position 63. On the other hand, the non-mutant XI12 AHAS gene cloned in XI12/17A is completely homologous to the corresponding B73/10-2 in the region coding for the mature AHAS protein (data not shown).

DEPOSIT OF BIOLOGICAL MATERIALS

The following biological materials were deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland, 20857, as follows:

E. coli XLI Blue harboring plasmid pX12/8A, deposited on July 3, 1991, Accession Number ATCC 68643

XI12 corn seed deposited on July 16, 1991, Accession Number ATCC 75051.

Sequence Listings

5 Sequence ID No.: 1

Sequence Type: Nucleotide and Amino Acid

10 Sequence Length: 1969 BP's and 638 Amino Acids

Strandedness: Single

15 Topology: Linear

20 Original Source Organism: Zea mays

Properties: Herbicide Resistant AHAS Enzyme

25
AACCCTCGCG CCGCCTCCGA GACAGCCGCC GCAACC 36

30
ATG GCC ACC GCC GCC GCC GCG TCT ACC GCG CTC ACT 72
Met Ala Thr Ala Ala Ala Ala Ser Thr Ala Leu Thr
1 5 10

35
GGC GCC ACT ACC GCT GCG CCC AAG GCG AGG CGC CGG 108
Gly Ala Thr Thr Ala Ala Pro Lys Ala Arg Arg Arg
15 20

40
GCG CAC CTC CTG GCC ACC CGC CGC GCC CTC GCC GCG 144
Ala His Leu Leu Ala Thr Arg Arg Ala Leu Ala Ala
25 30 35

45
CCC ATC AGG TGC TCA GCG GCG TCA CCC GCC ATG CCG 180
Pro Ile Arg Cys Ser Ala Ala Ser Pro Ala Met Pro
40 45

55

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5	ATG GCT CCC CCG GCC ACC CCG CTC CGG CCG TGG GGC	216
	Met Ala Pro Pro Ala Thr Pro Leu Arg Pro Trp Gly	
	50 55 60	
10	CCC ACC GAT CCC CGC AAG GGC GCC GAC ATC CTC GTC	252
	Pro Thr Asp Pro Arg Lys Gly Ala Asp Ile Leu Val	
	65 70	
15	GAG TCC CTC GAG CGC TGC GGC GTC CGC GAC GTC TTC	288
	Glu Ser Leu Glu Arg Cys Gly Val Arg Asp Val Phe	
	75 80	
20	GCC TAC CCC GGC GGC GCG TCC ATG GAG ATC CAC CAG	324
	Ala Tyr Pro Gly Gly Ala Ser Met Glu Ile His Gln	
25	85 90 95	
30	GCA CTC ACC CGC TCC CCC GTC ATC GCC AAC CAC CTC	360
	Ala Leu Thr Arg Ser Pro Val Ile Ala Asn His Leu	
	100 105	
35	TTC CGC CAC GAG CAA GGG GAG GCC TTT GCG GCC TCC	396
	Phe Arg His Glu Gln Gly Glu Ala Phe Ala Ala Ser	
	110 115 120	
40	GGC TAC GCG CGC TCC TCG GGC CGC GTC GGC GTC TGC	432
	Gly Tyr Ala Arg Ser Ser Gly Arg Val Gly Val Cys	
	125 130	
45	ATC GCC ACC TCC GGC CCC GGC GCC ACC AAC CTT GTC	468
	Ile Ala Thr Ser Gly Pro Gly Ala Thr Asn Leu Val	
	135 140	
50	TCC GCG CTC GCC GAC GCG CTG CTC GAT TCC GTC CCC	504
	Ser Ala Leu Ala Asp Ala Leu Leu Asp Ser Val Pro	
55	145 150 155	

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5	ATG GTC GCC ATC ACG GGA CAG GTG CCG CGA CGC ATG	540
	Met Val Ala Ile Thr Gly Gln Val Pro Arg Arg Met	
	160 165	
10	ATT GGC ACC GAC GCC TTC CAG GAG ACG CCC ATC GTC	576
	Ile Gly Thr Asp Ala Phe Gln Glu Thr Pro Ile Val	
	170 175 180	
15	GAG GTC ACC CGC TCC ATC ACC AAG CAC AAC TAC CTG	612
	Glu Val Thr Arg Ser Ile Thr Lys His Asn Tyr Leu	
	185 190	
20	GTC CTC GAC GTC GAC GAC ATC CCC CGC GTC GTG CAG	648
	Val Leu Asp Val Asp Asp Ile Pro Arg Val Val Gln	
25	195 200	
30	GAG GCT TTC TTC CTC GCC TCC TCT GGT CGA CCG GGG	684
	Glu Ala Phe Phe Leu Ala Ser Ser Gly Arg Pro Gly	
	205 210 215	
35	CCG GTG CTT GTC GAC ATC CCC AAG GAC ATC CAG CAG	720
	Pro Val Leu Val Asp Ile Pro Lys Asp Ile Gln Gln	
	220 225	
40	CAG ATG GCG GTG CCT GTC TGG GAC AAG CCC ATG AGT	756
	Gln Met Ala Val Pro Val Trp Asp Lys Pro Met Ser	
	230 235 240	
45	CTG CCT GGG TAC ATT GCG CGC CTT CCC AAG CCC CCT	792
	Leu Pro Gly Tyr Ile Ala Arg Leu Pro Lys Pro Pro	
	245 250	
50	GCG ACT GAG TTG CTT GAG CAG GTG CTG CGT CTT GTT	828
	Ala Thr Glu Leu Leu Glu Gln Val Leu Arg Leu Val	
55	255 260	

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5	GGT GAA TCC CGG CGC CCT GTT CTT TAT GTT GGC GGT	864
	Gly Glu Ser Arg Arg Pro Val Leu Tyr Val Gly Gly	
	265 270 275	
10	GCG TGC GCA GCA TCT GGT GAG GAG TTG CGA CGC TTT	900
	Ala Cys Ala Ala Ser Gly Glu Glu Leu Arg Arg Phe	
	280 285	
15	GTG GAG CTG ACT GGA ATC CCG GTC ACA ACT ACT CTT	936
	Val Glu Leu Thr Gly Ile Pro Val Thr Thr Thr Leu	
	290 295 300	
20	ATG GGC CTC GGC AAC TTC CCC AGC GAC GAC CCA CTG	972
	Met Gly Leu Gly Asn Phe Pro Ser Asp Asp Pro Leu	
	305 310	
25	TCT CTG CGC ATG CTA GGT ATG CAT GGC ACG GTG TAT	1008
	Ser Leu Arg Met Leu Gly Met His Gly Thr Val Tyr	
30	315 320	
35	GCA AAT TAT GCA GTG GAT AAG GCC GAT CTG TTG CTT	1044
	Ala Asn Tyr Ala Val Asp Lys Ala Asp Leu Leu Leu	
	325 330 335	
40	GCA CTT GGT GTG CGG TTT GAT GAT CGT GTG ACA GGG	1080
	Ala Leu Gly Val Arg Phe Asp Asp Arg Val Thr Gly	
	340 345	
45	AAG ATT GAG GCT TTT GCA AGC AGG GCT AAG ATT GTG	1116
	Lys Ile Glu Ala Phe Ala Ser Arg Ala Lys Ile Val	
	350 355 360	
50	CAC GTT GAT ATT GAT CCG GCT GAG ATT GGC AAG AAC	1152
	His Val Asp Ile Asp Pro Ala Glu Ile Gly Lys Asn	
55	365 370	

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	AAG CAG CCA CAT GTG TCC ATC TGT GCA GAT GTT AAG	1188
	Lys Gln Pro His Val Ser Ile Cys Ala Asp Val Lys	
5	375 380	
	CTT GCT TTG CAG GGC ATG AAT GCT CTT CTT GAA GGA	1224
	Leu Ala Leu Gln Gly Met Asn Ala Leu Leu Glu Gly	
10	385 390 395	
	AGC ACA TCA AAG AAG AGC TTT GAC TTT GGC TCA TGG	1260
	Ser Thr Ser Lys Lys Ser Phe Asp Phe Gly Ser Trp	
15	400 405	
	AAC GAT GAG TTG GAT CAG CAG AAG AGG GAA TTC CCC	1296
	Asn Asp Glu Leu Asp Gln Gln Lys Arg Glu Phe Pro	
20	410 415 420	
	CTT GGG TAT AAA ACA TCT AAT GAG GAG ATC CAG CCA	1332
	Leu Gly Tyr Lys Thr Ser Asn Glu Glu Ile Gln Pro	
25	425 430	
	CAA TAT GCT ATT CAG GTT CTT GAT GAG CTG ACG AAA	1368
	Gln Tyr Ala Ile Gln Val Leu Asp Glu Leu Thr Lys	
30	435 440	
	GGC GAG GCC ATC ATC GGC ACA GGT GTT GGG CAG CAC	1404
	Gly Glu Ala Ile Ile Gly Thr Gly Val Gly Gln His	
40	445 450 455	
	CAT ATG TGG GCG GCA CAG TAC TAC ACT TAC AAG CGG	1440
	Gln Met Trp Ala Ala Gln Tyr Tyr Thr Tyr Lys Arg	
45	460 465	
	CCA AGG CAG TGG TTG TCT TCA GCT GGT CTT GGG GCT	1476
	Pro Arg Gln Trp Leu Ser Ser Ala Gly Leu Gly Ala	
50	470 475 480	
55		

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	ATG GGA TTT GGT TTG CCG GCT GCT GCT GGT GCT TCT	1512
	Met Gly Phe Gly Leu Pro Ala Ala Ala Gly Ala Ser	
5	485 490	
	GTG GCC AAC CCA GGT GTT ACT GTT GTT GAC ATC GAT	1548
	Val Ala Asn Pro Gly Val Thr Val Val Asp Ile Asp	
10	495 500	
	GGA GAT GGT AGC TTT CTC ATG AAC GTT CAG GAG CTA	1584
	Gly Asp Gly Ser Phe Leu Met Asn Val Gln Glu Leu	
15	505 510 515	
	GCT ATG ATC CGA ATT GAG AAC CTC CCG GTG AAG GTC	1620
	Ala Met Ile Arg Ile Glu Asn Leu Pro Val Lys Val	
20	520 525	
	TTT GTG CTA AAC AAC CAG CAC CTG GGG ATG GTG GTG	1656
	Phe Val Leu Asn Asn Gln His Leu Gly Met Val Val	
25	530 535 540	
	CAG TGG GAG GAC AGG TTC TAT AAG GCC AAC AGA GCG	1692
	Gln Trp Glu Asp Arg Phe Tyr Lys Ala Asn Arg Ala	
30	545 550	
	CAC ACA TAC TTG GGA AAC CCA GAG AAT GAA AGT GAG	1728
	His Thr Tyr Leu Gly Asn Pro Glu Asn Glu Ser Glu	
35	555 560	
	ATA TAT CCA GAT TTC GTG ACG ATC GCC AAA GGG TTC	1764
	Ile Tyr Pro Asp Phe Val Thr Ile Ala Lys Gly Phe	
40	565 570 575	
	AAC ATT CCA GCG GTC CGT GTG ACA AAG AAG AAC GAA	1800
	Asn Ile Pro Ala Val Arg Val Thr Lys Lys Asn Glu	
45	580 585	
50		
55		

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	GTC CGC GCA GCG ATA AAG AAG ATG CTC GAG ACT CCA	1836
	Val Arg Ala Ala Ile Lys Lys Met Leu Glu Thr Pro	
5	590 595 600	
	GGG CCG TAC CTC TTG GAT ATA ATC GTC CCA CAC CAG	1872
10	Gly Pro Tyr Leu Leu Asp Ile Ile Val Pro His Gln	
	605 610	
	GAG CAT GTG TTG CCT ATG ATC CCT AAT GGT GGG GCT	1908
15	Glu His Val Leu Pro Met Ile Pro Asn Gly Gly Ala	
	615 620	
	TTC AAG GAT ATG ATC CTG GAT GGT GAT GGC AGG ACT	1944
20	Phe Lys Asp Met Ile Leu Asp Gly Asp Gly Arg Thr	
	625 630 635	
25	GTG TAC	1950
	Val Tyr	
	638	
30	TGATCTAAAA TCCAGCAAG	1969
35		
40		
45		
50		
55		

Sequence ID No.: 2

Sequence Type: Nucleotide and Amino Acid

Sequence Length: 1969 BP's and 638 Amino Acids

Strandedness: Single

Topology: Linear

Original Source Organism: Zea mays

Properties: Herbicide Sensitive AHAS Enzyme

AACCCTCGCG CCGCCTCCGA GACAGCCGCC GCAACC 36

ATG GCC ACC GCC GCC GCC GCG TCT ACC GCG CTC ACT 72
Met Ala Thr Ala Ala Ala Ala Ser Thr Ala Leu Thr
1 5 10

GCG GCC ACT ACC GCT GCG CCC AAG GCG AGG CGC CGG 108
Gly Ala Thr Thr Ala Ala Pro Lys Ala Arg Arg Arg
15 20

GCG CAC CTC CTG GCC ACC CGC CGC GCC CTC GCC GCG 144
Ala His Leu Leu Ala Thr Arg Arg Ala Leu Ala Ala
25 30 35

CCC ATC AGG TGC TCA GCG GCG TCA CCC GCC ATG CCG 180
Pro Ile Arg Cys Ser Ala Ala Ser Pro Ala Met Pro
40 45

ATG GCT CCC CCG GCC ACC CCG CTC CGG CCG TGG GGC 216
Met Ala Pro Pro Ala Thr Pro Leu Arg Pro Trp Gly
50 55 60

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5	CCC ACC GAT CCC CGC AAG GGC GCC GAC ATC CTC GTC Pro Thr Asp Pro Arg Lys Gly Ala Asp Ile Leu Val	252
	65 70	
10	GAG TCC CTC GAG CGC TGC GGC GTC CGC GAC GTC TTC Glu Ser Leu Glu Arg Cys Gly Val Arg Asp Val Phe	288
	75 80	
15	GCC TAC CCC GGC GGC GCG TCC ATG GAG ATC CAC CAG Ala Tyr Pro Gly Gly Ala Ser Met Glu Ile His Gln	324
	85 90 95	
20	GCA CTC ACC CGC TCC CCC GTC ATC GCC AAC CAC CTC Ala Leu Thr Arg Ser Pro Val Ile Ala Asn His Leu	360
	100 105	
25	TTC CGC CAC GAG CAA GGG GAG GCC TTT GCG GCC TCC Phe Arg His Glu Gln Gly Glu Ala Phe Ala Ala Ser	396
30	110 115 120	
35	GGC TAC GCG CGC TCC TCG GGC CGC GTC GGC GTC TGC Gly Tyr Ala Arg Ser Ser Gly Arg Val Gly Val Cys	432
	125 130	
40	ATC GCC ACC TCC GGC CCC GGC GCC ACC AAC CTT GTC Ile Ala Thr Ser Gly Pro Gly Ala Thr Asn Leu Val	468
	135 140	
45	TCC GCG CTC GCC GAC GCG CTG CTC GAT TCC GTC CCC Ser Ala Leu Ala Asp Ala Leu Leu Asp Ser Val Pro	504
	145 150 155	
50	ATG GTC GCC ATC ACG GGA CAG GTG CCG CGA CGC ATG Met Val Ala Ile Thr Gly Gln Val Pro Arg Arg Met	540
55	160 165	

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5	ATT GGC ACC GAC GCC TTC CAG GAG ACG CCC ATC GTC	576
	Ile Gly Thr Asp Ala Phe Gln Glu Thr Pro Ile Val	
	170 175 180	
10	GAG GTC ACC CGC TCC ATC ACC AAG CAC AAC TAC CTG	612
	Glu Val Thr Arg Ser Ile Thr Lys His Asn Tyr Leu	
	185 190	
15	GTC CTC GAC GTC GAC GAC ATC CCC CGC GTC GTG CAG	648
	Val Leu Asp Val Asp Asp Ile Pro Arg Val Val Gln	
	195 200	
20	GAG GCT TTC TTC CTC GCC TCC TCT GGT CGA CCG GGG	684
	Glu Ala Phe Phe Leu Ala Ser Ser Gly Arg Pro Gly	
25	205 210 215	
30	CCG GTG CTT GTC GAC ATC CCC AAG GAC ATC CAG CAG	720
	Pro Val Leu Val Asp Ile Pro Lys Asp Ile Gln Gln	
	220 225	
35	CAG ATG GCG GTG CCT GTC TGG GAC AAG CCC ATG AGT	756
	Gln Met Ala Val Pro Val Trp Asp Lys Pro Met Ser	
	230 235 240	
40	CTG CCT GGG TAC ATT GCG CGC CTT CCC AAG CCC CCT	792
	Leu Pro Gly Tyr Ile Ala Arg Leu Pro Lys Pro Pro	
	245 250	
45	GCG ACT GAG TTG CTT GAG CAG GTG CTG CGT CTT GTT	828
	Ala Thr Glu Leu Leu Glu Gln Val Leu Arg Leu Val	
	255 260	
50	GGT GAA TCC CGG CGC CCT GTT CTT TAT GTT GGC GGT	864
	Gly Glu Ser Arg Arg Pro Val Leu Tyr Val Gly Gly	
55	265 270 275	

5	GCG TGC GCA GCA TCT GGT GAG GAG TTG CGA CGC TTT	900
	Ala Cys Ala Ala Ser Gly Glu Glu Leu Arg Arg Phe	
	280 285	
10	GTG GAG CTG ACT GGA ATC CCG GTC ACA ACT ACT CTT	936
	Val Glu Leu Thr Gly Ile Pro Val Thr Thr Thr Leu	
	290 295 300	
15	ATG GGC CTC GGC AAC TTC CCC AGC GAC GAC CCA CTG	972
	Met Gly Leu Gly Asn Phe Pro Ser Asp Asp Pro Leu	
	305 310	
20	TCT CTG CGC ATG CTA GGT ATG CAT GGC ACG GTG TAT	1008
	Ser Leu Arg Met Leu Gly Met His Gly Thr Val Tyr	
25	315 320	
30	GCA AAT TAT GCA GTG GAT AAG GCC GAT CTG TTG CTT	1044
	Ala Asn Tyr Ala Val Asp Lys Ala Asp Leu Leu Leu	
	325 330 335	
35	GCA CTT GGT GTG CGG TTT GAT GAT CGT GTG ACA GGG	1080
	Ala Leu Gly Val Arg Phe Asp Asp Arg Val Thr Gly	
	340 345	
40	AAG ATT GAG GCT TTT GCA AGC AGG GCT AAG ATT GTG	1116
	Lys Ile Glu Ala Phe Ala Ser Arg Ala Lys Ile Val	
	350 355 360	
45	CAC GTT GAT ATT GAT CCG GCT GAG ATT GGC AAG AAC	1152
	His Val Asp Ile Asp Pro Ala Glu Ile Gly Lys Asn	
	365 370	
50	AAG CAG CCA CAT GTG TCC ATC TGT GCA GAT GTT AAG	1188
	Lys Gln Pro His Val Ser Ile Cys Ala Asp Val Lys	
55	375 380	

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5	CTT GCT TTG CAG GGC ATG AAT GCT CTT CTT GAA GGA	1224
	Leu Ala Leu Gln Gly Met Asn Ala Leu Leu Glu Gly	
	385 390 395	
10	AGC ACA TCA AAG AAG AGC TTT GAC TTT GGC TCA TGG	1260
	Ser Thr Ser Lys Lys Ser Phe Asp Phe Gly Ser Trp	
	400 405	
15	AAC GAT GAG TTG GAT CAG CAG AAG AGG GAA TTC CCC	1296
	Asn Asp Glu Leu Asp Gln Gln Lys Arg Glu Phe Pro	
	410 415 420	
20	CTT GGG TAT AAA ACA TCT AAT GAG GAG ATC CAG CCA	1332
	Leu Gly Tyr Lys Thr Ser Asn Glu Glu Ile Gln Pro	
	425 430	
25	CAA TAT GCT ATT CAG GTT CTT GAT GAG CTG ACG AAA	1368
	Gln Tyr Ala Ile Gln Val Leu Asp Glu Leu Thr Lys	
	435 440	
30	GGC GAG GCC ATC ATC GGC ACA GGT GTT GGG CAG CAC	1404
	Gly Glu Ala Ile Ile Gly Thr Gly Val Gly Gln His	
35	445 450 455	
40	CAT ATG TGG GCG GCA CAG TAC TAC ACT TAC AAG CGG	1440
	Gln Met Trp Ala Ala Gln Tyr Tyr Thr Tyr Lys Arg	
	460 465	
45	CCA AGG CAG TGG TTG TCT TCA GCT GGT CTT GGG GCT	1476
	Pro Arg Gln Trp Leu Ser Ser Ala Gly Leu Gly Ala	
	470 475 480	
50	ATG GGA TTT GGT TTG CCG GCT GCT GCT GGT GCT TCT	1512
	Met Gly Phe Gly Leu Pro Ala Ala Ala Gly Ala Ser	
	485 490	

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	GTG GCC AAC CCA GGT GTT ACT GTT GTT GAC ATC GAT	1548
	Val Ala Asn Pro Gly Val Thr Val Val Asp Ile Asp	
5	495 500	
	GGA GAT GGT AGC TTT CTC ATG AAC GTT CAG GAG CTA	1584
	Gly Asp Gly Ser Phe Leu Met Asn Val Gln Glu Leu	
10	505 510 515	
	GCT ATG ATC CGA ATT GAG AAC CTC CCG GTG AAG GTC	1620
	Ala Met Ile Arg Ile Glu Asn Leu Pro Val Lys Val	
15	520 525	
	TTT GTG CTA AAC AAC CAG CAC CTG GGG ATG GTG GTG	1656
	Phe Val Leu Asn Asn Gln His Leu Gly Met Val Val	
20	530 535 540	
	CAG TGG GAG GAC AGG TTC TAT AAG GCC AAC AGA GCG	1692
	Gln Trp Glu Asp Arg Phe Tyr Lys Ala Asn Arg Ala	
25	545 550	
	CAC ACA TAC TTG GGA AAC CCA GAG AAT GAA AGT GAG	1728
	His Thr Tyr Leu Gly Asn Pro Glu Asn Glu Ser Glu	
30	555 560	
	ATA TAT CCA GAT TTC GTG ACG ATC GCC AAA GGG TTC	1764
	Ile Tyr Pro Asp Phe Val Thr Ile Ala Lys Gly Phe	
35	565 570 575	
	AAC ATT CCA GCG GTC CGT GTG ACA AAG AAG AAC GAA	1800
	Asn Ile Pro Ala Val Arg Val Thr Lys Lys Asn Glu	
40	580 585	
	GTC CGC GCA GCG ATA AAG AAG ATG CTC GAG ACT CCA	1836
	Val Arg Ala Ala Ile Lys Lys Met Leu Glu Thr Pro	
45	590 595 600	
50		
55		

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	GGG CCG TAC CTC TTG GAT ATA ATC GTC CCA CAC CAG	1872
	Gly Pro Tyr Leu Leu Asp Ile Ile Val Pro His Gln	
5	605 610	
	GAG CAT GTG TTG CCT ATG ATC CCT AGT GGT GGG GCT	1908
10	Glu His Val Leu Pro Met Ile Pro Ser Gly Gly Ala	
	615 620	
	TTC AAG GAT ATG ATC CTG GAT GGT GAT GGC AGG ACT	1944
15	Phe Lys Asp Met Ile Leu Asp Gly Asp Gly Arg Thr	
	625 630 635	
	GTG TAC	1950
20	Val Tyr	
	638	
25	TGATCTAAAA TCCAGCAAG	1969
30		
35		
40		
45		
50		
55		

Sequence ID No.: 3

Sequence Type: Nucleotide and Amino Acid

Sequence Length: 1969 BP's and 638 Amino Acids

Strandedness: Single

Topology: Linear

Original Source Organism: Zea mays

Properties: Herbicide Sensitive AHAS Enzyme

AACCCTCGCG CCGCCTCCGA GACAGCCGCC GCAACC 36

ATG GCC ACC GCC GCC GCC GCG TCT ACC GCG CTC ACT 72

Met Ala Thr Ala Ala Ala Ala Ser Thr Ala Leu Thr

1 5 10

GGC GCC ACT ACC GCT GCG CCC AAG GCG AGG CGC CGG 108

Gly Ala Thr Thr Ala Ala Pro Lys Ala Arg Arg Arg

15 20

GCG CAC CTC CTG GCC ACC CGC CGC GCC CTC GCC GCG 144

Ala His Leu Leu Ala Thr Arg Arg Ala Leu Ala Ala

25 30 35

CCC ATC AGG TGC TCA GCG GCG TCA CCC GCC ATG CCG 180

Pro Ile Arg Cys Ser Ala Ala Ser Pro Ala Met Pro

40 45

ATG GCT CCC CCG GCC ACC CCG CTC CGG CCG TGG GGC 216

Met Ala Pro Pro Ala Thr Pro Leu Arg Pro Trp Gly

50 55 60

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5	CCC ACC GAG CCC CGC AAG GGT GCT GAC ATC CTC GTC	252
	Pro Thr Glu Pro Arg Lys Gly Ala Asp Ile Leu Val	
	65 70	
10	GAG TCC CTC GAG CGC TGC GGC GTC CGC GAC GTC TTC	288
	Glu Ser Leu Glu Arg Cys Gly Val Arg Asp Val Phe	
	75 80	
15	GCC TAC CCC GGC GGC GCG TCC ATG GAG ATC CAC CAG	324
	Ala Tyr Pro Gly Gly Ala Ser Met Glu Ile His Gln	
	85 90 95	
20	GCA CTC ACC CGC TCC CCC GTC ATC GCC AAC CAC CTC	360
	Ala Leu Thr Arg Ser Pro Val Ile Ala Asn His Leu	
	100 105	
25	TTC CGC CAC GAG CAA GGG GAG GCC TTT GCC GCC TCC	396
	Phe Arg His Glu Gln Gly Glu Ala Phe Ala Ala Ser	
30	110 115 120	
35	GGC TAC GCG CGC TCC TCG GGC CGC GTC GGC GTC TGC	432
	Gly Tyr Ala Arg Ser Ser Gly Arg Val Gly Val Cys	
	125 130	
40	ATC GCC ACC TCC GGC CCC GGC GCC ACC AAC CTA GTC	468
	Ile Ala Thr Ser Gly Pro Gly Ala Thr Asn Leu Val	
	135 140	
45	TCC GCG CTC GCC GAC GCG CTG CTC GAT TCC GTC CCC	504
	Ser Ala Leu Ala Asp Ala Leu Leu Asp Ser Val Pro	
	145 150 155	
50	ATG GTC GCC ATC ACG GGA CAG GTG CCG CGA CGC ATG	540
	Met Val Ala Ile Thr Gly Gln Val Pro Arg Arg Met	
	160 165	
55		

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	ATT GGC ACC GAC GCC TTC CAG GAG ACG CCC ATC GTC	576
	Trp Gly Thr Asp Ala Phe Gln Glu Thr Pro Ile Val	
5	170 175 180	
	GAG GTC ACC CGC TCC ATC ACC AAG CAC AAC TAC CTG	612
	Glu Val Thr Arg Ser Ile Thr Lys His Asn Tyr Leu	
10	185 190	
	GTC CTC GAC GTC GAC GAC ATC CCC CGC GTC GTG CAG	648
	Val Leu Asp Val Asp Asp Ile Pro Arg Val Val Gln	
15	195 200	
	GAG GCT TTC TTC CTC GCC TCC TCT GGT CGA CCA GGG	684
	Glu Ala Phe Phe Leu Ala Ser Ser Gly Arg Pro Gly	
20	205 210 215	
	CCG GTG CTT GTC GAC ATC CCC AAG GAC ATC CAG CAG	720
	Pro Val Leu Val Asp Ile Pro Lys Asp Ile Gln Gln	
25	220 225	
	CAG ATG GCG GTG CCT GTC TGG GAC AAG CCC ATG AGT	756
	Gln Met Ala Val Pro Val Trp Asp Lys Pro Met Ser	
30	230 235 240	
	CTG CCT GGG TAC ATT GCG CGC CTT CCC AAG CCC CCT	792
	Leu Pro Gly Tyr Ile Ala Arg Leu Pro Lys Pro Pro	
35	245 250	
	GCG ACT GAG TTG CTT GAG CAG GTG CTG CGT CTT GTT	828
	Ala Thr Glu Leu Leu Glu Gln Val Leu Arg Leu Val	
40	255 260	
	GGT GAA TCG CGG CGC CCT GTT CTT TAT GTG GGC GGT	864
	Gly Glu Ser Arg Arg Pro Val Leu Tyr Val Gly Gly	
45	265 270 275	
50		
55		

EP 0 525 384 A2

	GCG TGC GCA GCA TCT GGT GAG GAG TTG CGA CGC TTT	900
	Ala Cys Ala Ala Ser Gly Glu Glu Leu Arg Arg Phe	
5	280 285	
	GTG GAG CTG ACT GGA ATC CCG GTC ACA ACT ACT CTT	936
	Val Glu Leu Thr Gly Ile Pro Val Thr Thr Thr Leu	
10	290 295 300	
	ATG GGC CTC GGC AAC TTC CCC AGC GAC GAC CCA CTG	972
	Met Gly Leu Gly Asn Phe Pro Ser Asp Asp Pro Leu	
15	305 310	
	TCT CTG CGC ATG CTA GGT ATG CAT GGG ACG GTG TAT	1008
	Ser Leu Arg Met Leu Gly Met His Gly Thr Val Tyr	
20	315 320	
	GCA AAT TAT GCA GTG GAT AAG GCC GAT CTG TTG CTT	1044
	Ala Asn Tyr Ala Val Asp Lys Ala Asp Leu Leu Leu	
25	325 330 335	
	GCA CTT GGT GTG CGG TTT GAT GAT CGT GTG ACA GGG	1080
	Ala Leu Gly Val Arg Phe Asp Asp Arg Val Thr Gly	
30	340 345	
	AAG ATT GAG GCT TTT GCA AGC AGG GCT AAG ATT GTG	1116
	Lys Ile Glu Ala Phe Ala Ser Arg Ala Lys Ile Val	
35	350 355 360	
	CAC GTT GAT ATT GAT CCG GCT GAG ATT GGC AAG AAC	1152
	His Val Asp Ile Asp Pro Ala Glu Ile Gly Lys Asn	
40	365 370	
	AAG CAG CCA CAT GTG TCC ATC TGT GCA GAT GTT AAG	1188
	Lys Gln Pro His Val Ser Ile Cys Ala Asp Val Lys	
45	375 380	
50		
55		

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[illegible]

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5	GTG GCC AAC CCA GGT GTC ACT GTT GTT GAC ATC GAT	1548
	Val Ala Asn Pro Gly Val Thr Val Val Asp Ile Asp	
	495 500	
10	GGA GAT GGT AGC TTT CTC ATG AAC GTT CAG GAG CTA	1584
	Gly Asp Gly Ser Phe Leu Met Asn Val Gln Glu Leu	
	505 510 515	
15	GCT ATG ATC CGA ATT GAG AAC CTC CCA GTG AAG GTC	1620
	Ala Met Ile Arg Ile Glu Asn Leu Pro Val Lys Val	
	520 525	
20	TTT GTG CTA AAC AAC CAG CAC CTG GGG ATG GTG GTG	1656
	Phe Val Leu Asn Asn Gln His Leu Gly Met Val Val	
25	530 535 540	
30	CAG TGG GAG GAC AGG TTC TAT AAG GCC AAC AGA GCG	1692
	Gln Trp Glu Asp Arg Phe Tyr Lys Ala Asn Arg Ala	
	545 550	
35	CAC ACA TAC TTG GGA AAC CCA GAG AAT GAA AGT GAG	1728
	His Thr Tyr Leu Gly Asn Pro Glu Asn Glu Ser Glu	
	555 560	
40	ATA TAT CCA GAT TTC GTG ACG ATC GCC AAA GGG TTC	1764
	Ile Tyr Pro Asp Phe Val Thr Ile Ala Lys Gly Phe	
	565 570 575	
45	AAC ATT CCA GCG GTC CGT GTG ACA AAG AAG AAC GAA	1800
	Asn Ile Pro Ala Val Arg Val Thr Lys Lys Asn Glu	
	580 585	
50	GTC CGC GCA GCG ATA AAG AAG ATG CTC GAG ACT CCA	1836
	Val Arg Ala Ala Ile Lys Lys Met Leu Glu Thr Pro	
55	590 595 600	

5 GGG CCG TAC CTC TTG GAT ATA ATC GTC CCA CAC CAG 1872
Gly Pro Tyr Leu Leu Asp Ile Ile Val Pro His Gln
605 610

10 GAG CAT GTG TTG CCT ATG ATC CCT AGT GGT GGG GCT 1908
Glu His Val Leu Pro Met Ile Pro Ser Gly Gly Ala
615 620

15 TTC AAG GAT ATG ATC CTG GAT GGT GAT GGC AGG ACT 1944
Phe Lys Asp Met Ile Leu Asp Gly Asp Gly Arg Thr
625 630 635

20 GTG TAC 1950
Val Tyr
638

25 TGATCTAAAA TCCAGCAAG 1969

Claims

- 35 1. A monocot nucleic acid sequence encoding a functional AHAS enzyme, which enzyme has an amino acid substitution relative to a wild-type monocot AHAS enzyme, and which substitution confers imidazolinone-specific resistance to the enzyme.
- 40 2. The sequence of Claim 1 in which the monocot is corn and the substitution is at position 621 in the wild-type corn AHAS enzyme.
3. The sequence of Claim 2 in which the substituted amino acid is asparagine.
4. A functional monocot AHAS enzyme which has an amino acid substitution relative to a monocot wild-type AHAS enzyme, and which substitution confers imidazolinone-specific resistance to the enzyme.
- 45 5. The enzyme of Claim 4 in which the monocot is corn and the substitution is at position 621 in the wild-type corn AHAS enzyme.
- 50 6. The enzyme of Claim 5 in which the substituted amino acid is asparagine.
7. A transformation vector comprising the nucleic acid of Claim 1.
8. A host cell comprising the nucleic acid sequence of Claim 1, or the vector of Claim 7.
- 55 9. The host cell of Claim 8 which is a plant cell or a bacterial cell.
10. An imidazolinone-specific resistant mature plant containing the nucleic acid sequence of Claim 1, or seed or pollen therefrom.

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11. A method of conferring imidazolinone-specific resistance to a plant cell which comprises providing the plant cell with the nucleic acid sequence of Claim 1.
12. A method for growing imidazolinone-specific resistant plants which comprises cultivating a plant which produces the enzyme of Claim 4 in the presence of an inhibitory amount of imidazolinone.
13. A method of selecting host cells successfully transformed with a gene of interest which comprises providing to prospective host cells the gene of interest linked to the nucleic acid sequence of Claim 1, or unlinked but in the presence of the nucleic acid sequence of Claim 1, growing the cells in the presence of an inhibitory amount of imidazolinone and identifying surviving cells as containing the gene of interest.
14. A nucleic acid construct comprising the sequence of Claim 1 linked to a gene encoding an agronomically useful trait.

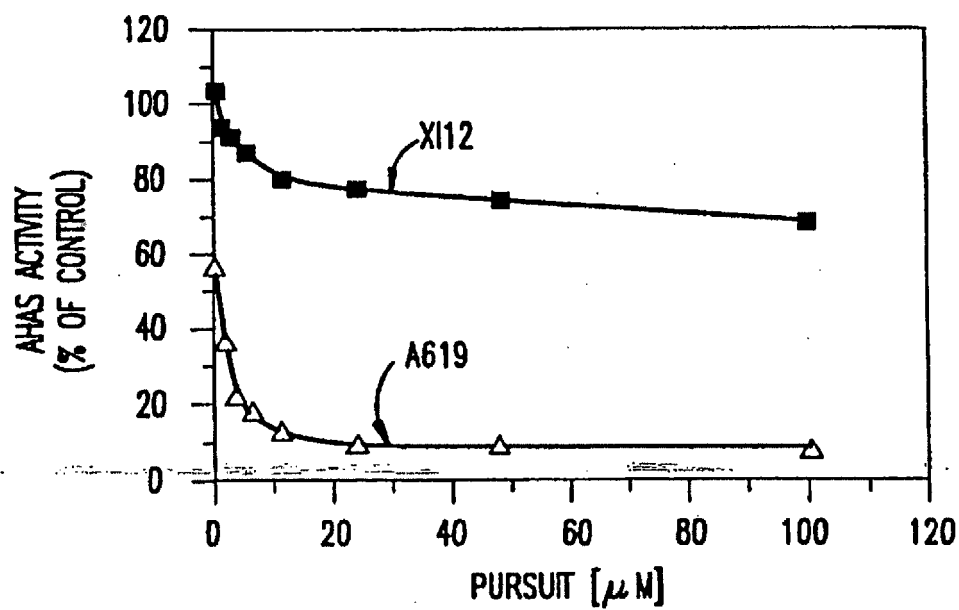


FIG. 1A

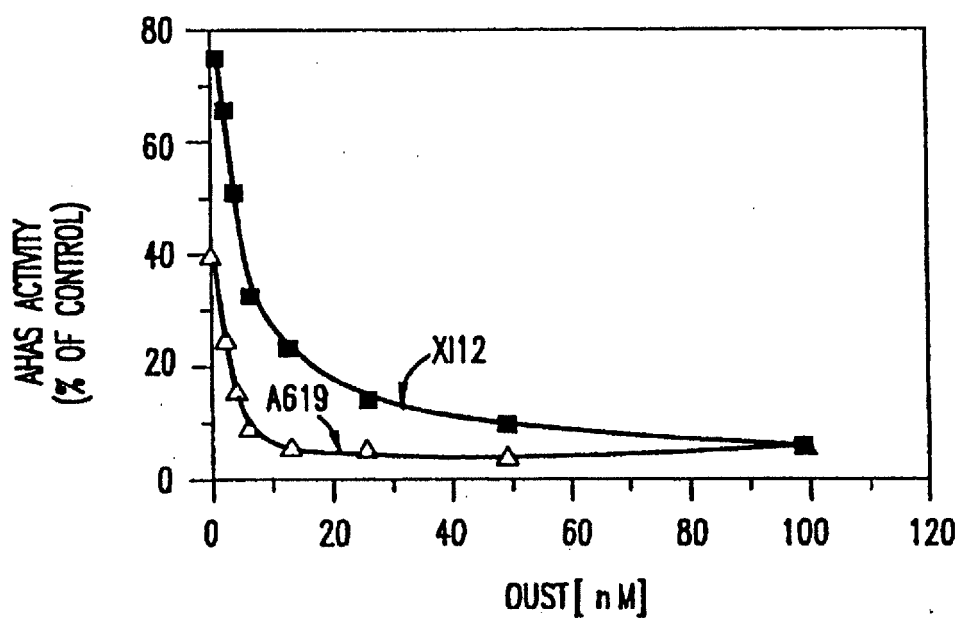


FIG. 1B

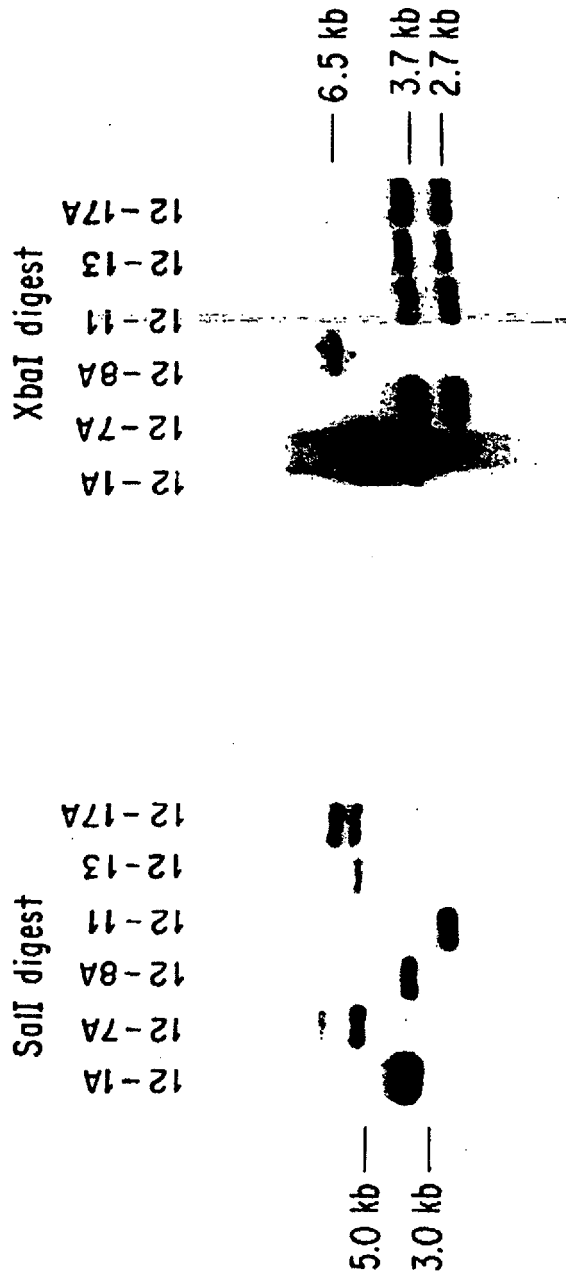


FIG. 2A

FIG. 2B

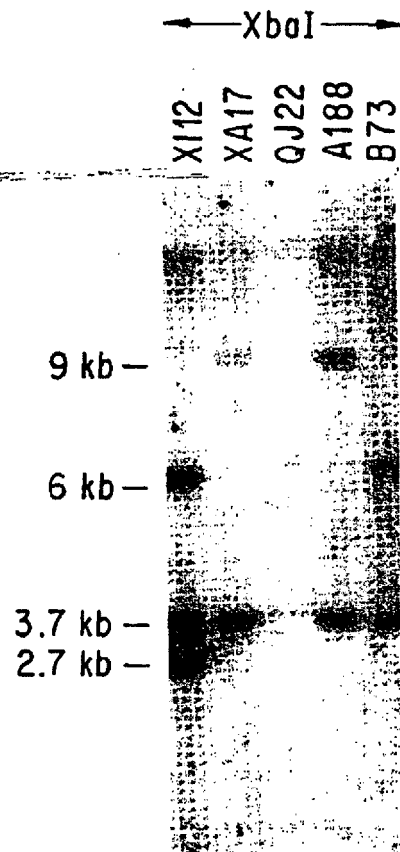


FIG. 3

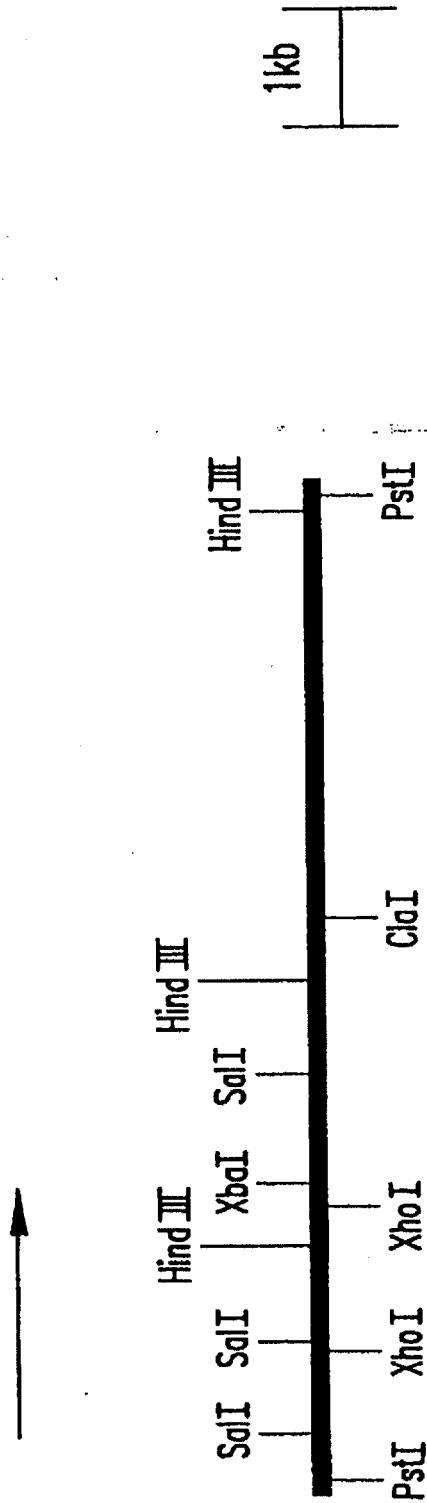


FIG.4

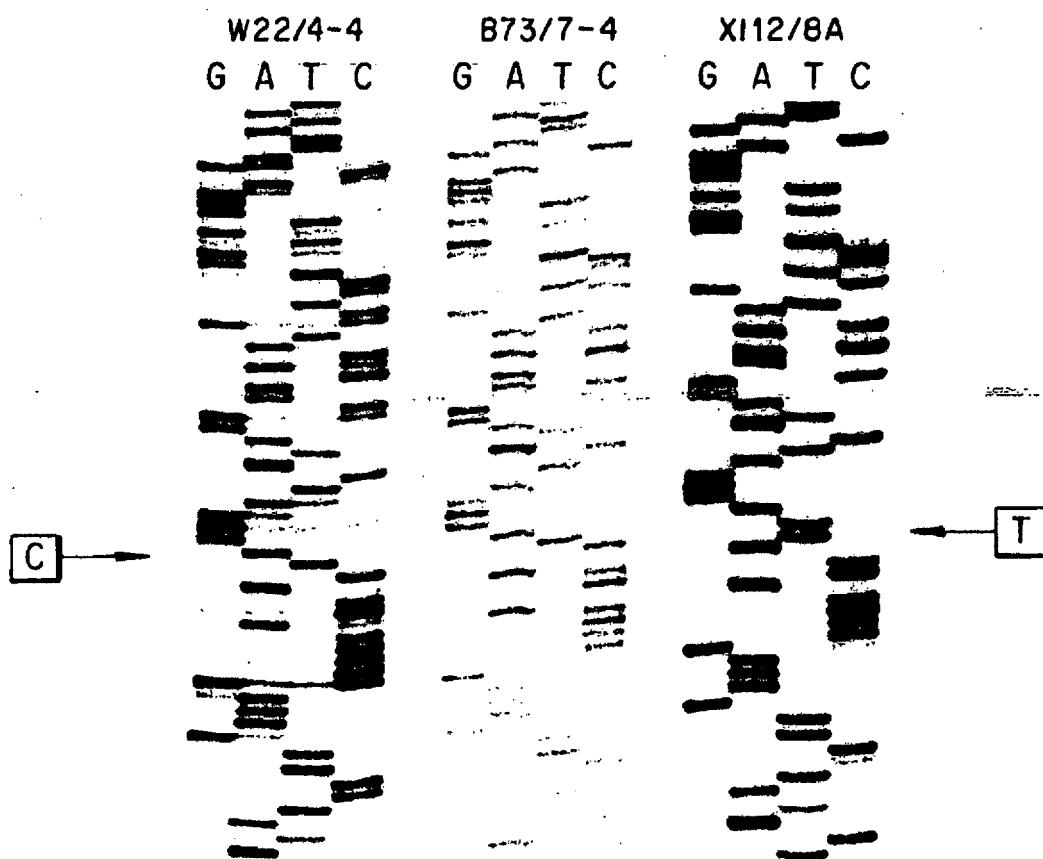


FIG. 5A

W22/1A and B73/7-4
sequence:

5'TAGTG3'
3'ATCTG5'

XI12/8A sequence:

5'TAATG3'
3'ATTAC5'

FIG. 5B

40	50	60	70	80	90
*	*	*	*	*	*
ATG GCC ACC GCC GCC GCC GCG TCT ACC GCG CTC ACT GGC GCC ACT ACC GCT GCG					
Met Ala Thr Ala Ala Ala Ala Ser Thr Ala Leu Thr Gly Ala Thr Thr Ala Ala					
100	110	120	130	140	
*	*	*	*	*	
CCC AAG GCG AGG CGC CGG GCG CAC CTC CTG GCC ACC CGC CGC GCC CTC GCC GCG					
Pro Lys Ala Arg Arg Arg Ala His Leu Leu Ala Thr Arg Arg Ala Leu Ala Ala					
150	160	170	180	190	
*	*	*	*	*	
CCC ATC AGG TGC TCA GCG GCG TCA CCC GCC ATG CCG ATG GCT CCC CCG GCC ACC					
Pro Ile Arg Cys Ser Ala Ala Ser Pro Ala Met Pro Met Ala Pro Pro Ala Thr					
200	210	220	230	240	250
*	*	*	*	*	*
CCG CTC CCG CCG TGG GGC CCC ACC GAT CCC CGC AAG GGC GCC GAC ATC CTC GTC					
Pro Leu Arg Pro Trp Gly Pro Thr Asp Pro Arg Lys Gly Ala Asp Ile Leu Val					
260	270	280	290	300	
*	*	*	*	*	
GAG TCC CTC GAG CGC TGC GGC GTC CGC GAC GTC TTC GCC TAC CCC GGC GGC GCG					
Glu Ser Leu Glu Arg Cys Gly Val Arg Asp Val Phe Ala Tyr Pro Gly Gly Ala					
310	320	330	340	350	360
*	*	*	*	*	*
TCC ATG GAG ATC CAC CAG GCA CTC ACC CGC TCC CCC GTC ATC GCC AAC CAC CTC					
Ser Met Glu Ile His Gln Ala Leu Thr Arg Ser Pro Val Ile Ala Asn His Leu					
370	380	390	400	410	
*	*	*	*	*	
TTC CGC CAC GAG CAA GGG GAG GCC TTT GCG GCC TCC GGC TAC GCG CGC TCC TCG					
Phe Arg His Glu Gln Gly Glu Ala Phe Ala Ala Ser Gly Tyr Ala Arg Ser Ser					

FIG.6A

420	430	440	450	460	
* * *	* * *	* * *	* * *	* * *	
GGC CGC GTC GGC GTC TGC ATC GCC ACC TCC GGC CCC GGC GCC ACC AAC CTT GTC					
Gly Arg Val Gly Val Cys Ile Ala Thr Ser Gly Pro Gly Ala Thr Asn Leu Val					
470	480	490	500	510	520
* * *	* * *	* * *	* * *	* * *	* * *
TCC GCG CTC GCC GAC GCG CTG CTC GAT TCC GTC CCC ATG GTC GCC ATC ACG GGA					
Ser Ala Leu Ala Asp Ala Leu Leu Asp Ser Val Pro Met Val Ala Ile Thr Gly					
530	540	550	560	570	
* * *	* * *	* * *	* * *	* * *	
CAG GTG CCG CGA CGC ATG ATT GGC ACC GAC GCC TTC CAG GAG ACG CCC ATC GTC					
Gln Val Pro Arg Arg Met Ile Gly Thr Asp Ala Phe Gln Glu Thr Pro Ile Val					
580	590	600	610	620	630
* * *	* * *	* * *	* * *	* * *	* * *
GAG GTC ACC CGC TCC ATC ACC AAG CAC AAC TAC CTG GTC CTC GAC GTC GAC GAC					
Glu Val Thr Arg Ser Ile Thr Lys His Asn Tyr Leu Val Leu Asp Val Asp Asp					
640	650	660	670	680	
* * *	* * *	* * *	* * *	* * *	
ATC CCC CGC GTC GTG CAG GAG GCT TTC TTC CTC GCC TCC TCT GGT CGA CCG GGG					
Ile Pro Arg Val Val Gln Glu Ala Phe Phe Leu Ala Ser Ser Gly Arg Pro Gly					
690	700	710	720	730	
* * *	* * *	* * *	* * *	* * *	
CCG GTG CTT GTC GAC ATC CCC AAG GAC ATC CAG CAG CAG ATG GCG GTG CCT GTC					
Pro Val Leu Val Asp Ile Pro Lys Asp Ile Gln Gln Gln Met Ala Val Pro Val					
740	750	760	770	780	790
* * *	* * *	* * *	* * *	* * *	* * *
TGG GAC AAG CCC ATG AGT CTG CCT GGG TAC ATT GCG CGC CTT CCC AAG CCC CCT					
Trp Asp Lys Pro Met Ser Leu Pro Gly Tyr Ile Ala Arg Leu Pro Lys Pro Pro					

FIG.6B

800	810	820	830	840	
*	*	*	*	*	
GCG ACT GAG TTG CTT GAG CAG GTG CTG CGT CTT GTT GGT GAA TCC CGG CGC CCT					
Ala Thr Glu Leu Leu Glu Gln Val Leu Arg Leu Val Gly Glu Ser Arg Arg Pro					
850	860	870	880	890	900
*	*	*	*	*	*
GTT CTT TAT GTT GGC GGT GCG TGC GCA GCA TCT GGT GAG GAG TTG CGA CGC TTT					
Val Leu Tyr Val Gly Gly Ala Cys Ala Ala Ser Gly Glu Glu Leu Arg Arg Phe					
910	920	930	940	950	
*	*	*	*	*	
GTG GAG CTG ACT GGA ATC CCG GTC ACA ACT ACT CTT ATG GGC CTC GGC AAC TTC					
Val Glu Leu Thr Gly Ile Pro Val Thr Thr Thr Leu Met Gly Leu Gly Asn Phe					
960	970	980	990	1000	
*	*	*	*	*	
CCC AGC GAC GAC CCA CTG TCT CTG CGC ATG CTA GGT ATG CAT GGC ACG GTG TAT					
Pro Ser Asp Asp Pro Leu Ser Leu Arg Met Leu Gly Met His Gly Thr Val Tyr					
1010	1020	1030	1040	1050	1060
*	*	*	*	*	*
GCA AAT TAT GCA GTG GAT AAG GCC GAT CTG TTG CTT GCA CTT GGT GTG CGG TTT					
Ala Asn Tyr Ala Val Asp Lys Ala Asp Leu Leu Leu Ala Leu Gly Val Arg Phe					
1070	1080	1090	1100	1110	
*	*	*	*	*	
GAT GAT CGT GTG ACA GGG AAG ATT GAG GCT TTT GCA AGC AGG GCT AAG ATT GTG					
Asp Asp Arg Val Thr Gly Lys Ile Glu Ala Phe Ala Ser Arg Ala Lys Ile Val					
1120	1130	1140	1150	1160	1170
*	*	*	*	*	*
CAC GTT GAT ATT GAT CCG GCT GAG ATT GGC AAG AAC AAG CAG CCA CAT GTG TCC					
His Val Asp Ile Asp Pro Ala Glu Ile Gly Lys Asn Lys Gln Pro His Val Ser					

FIG.6C

1180	1190	1200	1210	1220	
ATC TGT GCA GAT GTT AAG CTT GCT TTG CAG GGC ATG AAT GCT CTT CTT GAA GGA					
Ile Cys Ala Asp Val Lys Leu Ala Leu Gln Gly Met Asn Ala Leu Leu Glu Gly					
1230	1240	1250	1260	1270	
AGC ACA TCA AAG AAG AGC TTT GAC TTT GGC TCA TGG AAC GAT GAG TTG GAT CAG					
Ser Thr Ser Lys Lys Ser Phe Asp Phe Gly Ser Trp Asn Asp Glu Leu Asp Gln					
1280	1290	1300	1310	1320	1330
CAG AAG AGG GAA TTC CCC CTT GGG TAT AAA ACA TCT AAT GAG GAG ATC CAG CCA					
Gln Lys Arg Glu Phe Pro Leu Gly Tyr Lys Thr Ser Asn Glu Glu Ile Gln Pro					
1340	1350	1360	1370	1380	
CAA TAT GCT ATT CAG GTT CTT GAT GAG CTG ACG AAA GGC GAG GCC ATC ATC GGC					
Gln Tyr Ala Ile Gln Val Leu Asp Glu Leu Thr Lys Gly Glu Ala Ile Ile Gly					
1390	1400	1410	1420	1430	1440
ACA GGT GTT GGG CAG CAC CAG ATG TGG GCG GCA CAG TAC TAC ACT TAC AAG CGG					
Thr Gly Val Gly Gln His Gln Met Trp Ala Ala Gln Tyr Tyr Thr Tyr Lys Arg					
1450	1460	1470	1480	1490	
CCA AGG CAG TGG TTG TCT TCA GCT GGT CTT GGG GCT ATG GGA TTT GGT TTG CCG					
Pro Arg Gln Trp Leu Ser Ser Ala Gly Leu Gly Ala Met Gly Phe Gly Leu Pro					
1500	1510	1520	1530	1540	
GCT GCT GCT GGT GCT TCT GTG GCC AAC CCA GGT GTT ACT GTT GTT GAC ATC GAT					
Ala Ala Ala Gly Ala Ser Val Ala Asn Pro Gly Val Thr Val Val Asp Ile Asp					
1550	1560	1570	1580	1590	1600
GGA GAT GGT AGC TTT CTC ATG AAC GTT CAG GAG CTA GCT ATG ATC CGA ATT GAG					
Gly Asp Gly Ser Phe Leu Met Asn Val Gln Glu Leu Ala Met Ile Arg Ile Glu					

FIG.6D

1610	1620	1630	1640	1650	
⌘	⌘	⌘	⌘	⌘	
AAC CTC CCG GTG AAG GTC TTT GTG CTA AAC AAC CAG CAC CTG GGG ATG GTG GTG					
Asn Leu Pro Val Lys Val Phe Val Leu Asn Asn Gln His Leu Gly Met Val Val					
1660	1670	1680	1690	1700	1710
⌘	⌘	⌘	⌘	⌘	⌘
CAG TGG GAG GAC AGG TTC TAT AAG GCC AAC AGA GCG CAC ACA TAC TTG GGA AAC					
Gln Trp Glu Asp Arg Phe Tyr Lys Ala Asn Arg Ala His Thr Tyr Leu Gly Asn					
1720	1730	1740	1750	1760	
⌘	⌘	⌘	⌘	⌘	
CCA GAG AAT GAA AGT GAG ATA TAT CCA GAT TTC GTG ACG ATC GCC AAA GGG TTC					
Pro Glu Asn Glu Ser Glu Ile Tyr Pro Asp Phe Val Thr Ile Ala Lys Gly Phe					
1770	1780	1790	1800	1810	
⌘	⌘	⌘	⌘	⌘	
AAC ATT CCA GCG GTC CGT GTG ACA AAG AAG AAC GAA GTC CGC GCA GCG ATA AAG					
Asn Ile Pro Ala Val Arg Val Thr Lys Lys Asn Glu Val Arg Ala Ala Ile Lys					
1820	1830	1840	1850	1860	1870
⌘	⌘	⌘	⌘	⌘	⌘
AAG ATG CTC GAG ACT CCA GGG CCG TAC CTC TTG GAT ATA ATC GTC CCA CAC CAG					
Lys Met Leu Glu Thr Pro Gly Pro Tyr Leu Leu Asp Ile Ile Val Pro His Gln					
1880	1890	1900	1910	1920	
⌘	⌘	⌘	⌘	⌘	
GAG CAT GTG TTG CCT ATG ATC CCT AAT GGT GGG GCT TTC AAG GAT ATG ATC CTG					
Glu His Val Leu Pro Met Ile Pro Asn Gly Gly Ala Phe Lys Asp Met Ile Leu					
1930	1940	1950	1960		
⌘	⌘	⌘	⌘		
GAT GGT GAT GGC AGG ACT GTG TAC TGATC TAAAA TCCAG CAAG					
Asp Gly Asp Gly Arg Thr Val Tyr					

FIG.6E

	10	20	30	40	50	60
XI12/8A	AACCC TCGCG CCGCC TCCGA GACAG CCGCC GCAAC CATGG CCACC GCCGC CGCCG CGTCT					
W22/1A	AACCC TCGCG CCGCC TCCGA GACAG CCGCC GCAAC CATGG CCACC GCCGC CGCCG CGTCT					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
B73/7-4	AACCC TCGCG CCGCC TCCGA GACAG CCGCC GCAAC CATGG CCACC GCCGC CGCCG CGTCT					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
XI12/8A	AACCC TCGCG CCGCC TCCGA GACAG CCGCC GCAAC CATGG CCACC GCCGC CGCCG CGTCT					
	70	80	90	100	110	120
XI12/8A	ACCGC GCTCA CTGGC GCCAC TACCG CTGCG CCCAA GGCGA GCGGC CGGGC GCACC TCCTG					
W22/1A	ACCGC GCTCA CTGGC GCCAC TACCG CTGCG CCCAA GGCGA GCGGC CGGGC GCACC TCCTG					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
B73/7-4	ACCGC GCTCA CTGGC GCCAC TACCG CTGCG CCCAA GGCGA GCGGC CGGGC GCACC TCCTG					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
XI12/8A	ACCGC GCTCA CTGGC GCCAC TACCG CTGCG CCCAA GGCGA GCGGC CGGGC GCACC TCCTG					
	130	140	150	160	170	180
XI12/8A	GCCAC CCGCC GCGCC CTCGC CGCGC CCATC AGGTG CTCAG CGGCG TCACC CGCCA TGCCG					
W22/1A	GCCAC CCGCC GCGCC CTCGC CGCGC CCATC AGGTG CTCAG CGGCG TCACC CGCCA TGCCG					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
B73/7-4	GCCAC CCGCC GCGCC CTCGC CGCGC CCATC AGGTG CTCAG CGGCG TCACC CGCCA TGCCG					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
XI12/8A	GCCAC CCGCC GCGCC CTCGC CGCGC CCATC AGGTG CTCAG CGGCG TCACC CGCCA TGCCG					
	190	200	210	220	230	240
XI12/8A	ATGGC TCCCC CGGCC ACCCC GCTCC GGCCG TGGGG CCCCA CCGAT CCCCC CAAGG GCGCC					
					#	> >
W22/1A	ATGGC TCCCC CGGCC ACCCC GCTCC GGCCG TGGGG CCCCA CCGAT CCCCC CAAGG GCGCC					
	11111 11111 11111 11111 11111 11111 11111 11111 1111 11111 11111 1 11					
B73/7-4	ATGGC TCCCC CGGCC ACCCC GCTCC GGCCG TGGGG CCCCA CCGAg CCCCC CAAGG GtGct					
	11111 11111 11111 11111 11111 11111 11111 11111 1111 11111 11111 1 11					
XI12/8A	ATGGC TCCCC CGGCC ACCCC GCTCC GGCCG TGGGG CCCCA CCGAT CCCCC CAAGG GCGCC					

FIG.7A

	250	260	270	280	290	300
XI12/8A	GACAT CCTCG TCGAG TCCCT CGAGC GCTGC GGCCT CCGCG ACGTC TTCGC CTACC CCGGC					
W22/1A	GACAT CCTCG TCGAG TCCCT CGAGC GCTGC GGCCT CCGCG ACGTC TTCGC CTACC CCGGC					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
B73/7-4	GACAT CCTCG TCGAG TCCCT CGAGC GCTGC GGCCT CCGCG ACGTC TTCGC CTACC CCGGC					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
XI12/8A	GACAT CCTCG TCGAG TCCCT CGAGC GCTGC GGCCT CCGCG ACGTC TTCGC CTACC CCGGC					

	310	320	330	340	350	360
XI12/8A	GGCGC GTCCA TGGAG ATCCA CCAGG CACTC ACCCG CTCCC CCGTC ATCGC CAACC ACCTC					
W22/1A	GGCGC GTCCA TGGAG ATCCA CCAGG CACTC ACCCG CTCCC CCGTC ATCGC CAACC ACCTC					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
XB73/7-4	GGCGC GTCCA TGGAG ATCCA CCAGG CACTC ACCCG CTCCC CCGTC ATCGC CAACC ACCTC					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
XI12/8A	GGCGC GTCCA TGGAG ATCCA CCAGG CACTC ACCCG CTCCC CCGTC ATCGC CAACC ACCTC					

	370	380	390	400	410	420
XI12/8A	TTCCG CCACG AGCAA GGGGA GGCCT TTGCG GCCTC CGGCT ACGCG CGCTC CTCGG GCCGC					
W22/1A	TTCCG CCACG AGCAA GGGGA GGCCT TTGCG GCCTC CGGCT ACGCG CGCTC CTCGG GCCGC					
	11111 11111 11111 11111 11111 1111 11111 11111 11111 11111 11111 11111					
B73/7-4	TTCCG CCACG AGCAA GGGGA GGCCT TTGCG GCCTC CGGCT ACGCG CGCTC CTCGG GCCGC					
	11111 11111 11111 11111 11111 1111 11111 11111 11111 11111 11111 11111					
XI12/8A	TTCCG CCACG AGCAA GGGGA GGCCT TTGCG GCCTC CGGCT ACGCG CGCTC CTCGG GCCGC					

	430	440	450	460	470	480
XI12/8A	GTCGG CGTCT GCATC GCCAC CTCGG GCCCC GGC GC CACCA ACCTT GTCTC CGCGC TCGCC					
W22/1A	GTCGG CGTCT GCATC GCCAC CTCGG GCCCC GGC GC CACCA ACCTT GTCTC CGCGC TCGCC					
	11111 11111 11111 11111 11111 11111 11111 11111 1111 11111 11111 11111					
B73/7-4	GTCGG CGTCT GCATC GCCAC CTCGG GCCCC GGC GC CACCA ACCTT GTCTC CGCGC TCGCC					
	11111 11111 11111 11111 11111 11111 11111 11111 1111 11111 11111 11111					
XI12/8A	GTCGG CGTCT GCATC GCCAC CTCGG GCCCC GGC GC CACCA ACCTT GTCTC CGCGC TCGCC					

FIG.7B

	490	500	510	520	530	540
XI12/8A	GACGC GCTGC TCGAT TCCGT CCCCC TGGTC GCCAT CACGG GACAG GTGCC GCGAC GCATG					
V22/1A	GACGC GCTGC TCGAT TCCGT CCCCC TGGTC GCCAT CACGG GACAG GTGCC GCGAC GCATG					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
B73/7-4	GACGC GCTGC TCGAT TCCGT CCCCC TGGTC GCCAT CACGG GACAG GTGCC GCGAC GCATG					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
XI12/8A	GACGC GCTGC TCGAT TCCGT CCCCC TGGTC GCCAT CACGG GACAG GTGCC GCGAC GCATG					
	550	560	570	580	590	600
XI12/8A	ATTGG CACCG ACGCC TTCCA GGAGA CGCCC ATCGT CGAGG TCACC CGCTC CATCA CCAAG					
V22/1A	ATTGG CACCG ACGCC TTCCA GGAGA CGCCC ATCGT CGAGG TCACC CGCTC CATCA CCAAG					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
B73/7-4	ATTGG CACCG ACGCC TTCCA GGAGA CGCCC ATCGT CGAGG TCACC CGCTC CATCA CCAAG					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
XI12/8A	ATTGG CACCG ACGCC TTCCA GGAGA CGCCC ATCGT CGAGG TCACC CGCTC CATCA CCAAG					
	610	620	630	640	650	660
XI12/8A	CACAA CTACC TGGTC CTCGA CGTCG ACGAC ATCCC CCGCG TCGTG CAGGA GGCTT TCTTC					
V22/1A	CACAA CTACC TGGTC CTCGA CGTCG ACGAC ATCCC CCGCG TCGTG CAGGA GGCTT TCTTC					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
B73/7-4	CACAA CTACC TGGTC CTCGA CGTCG ACGAC ATCCC CCGCG TCGTG CAGGA GGCTT TCTTC					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
XI12/8A	CACAA CTACC TGGTC CTCGA CGTCG ACGAC ATCCC CCGCG TCGTG CAGGA GGCTT TCTTC					
	670	680	690	700	710	720
XI12/8A	CTCGC CTCCT CTGGT CGACC GGGGC CGGTG CTTGT CGACA TCCCC AAGGA CATCC AGCAG					
V22/1A	CTCGC CTCCT CTGGT CGACC GGGGC CGGTG CTTGT CGACA TCCCC AAGGA CATCC AGCAG					
	11111 11111 11111 11111 1111 11111 11111 11111 11111 11111 11111 11111					
B73/7-4	CTCGC CTCCT CTGGT CGACC GGGGC CGGTG CTTGT CGACA TCCCC AAGGA CATCC AGCAG					
	11111 11111 11111 11111 1111 11111 11111 11111 11111 11111 11111 11111					
XI12/8A	CTCGC CTCCT CTGGT CGACC GGGGC CGGTG CTTGT CGACA TCCCC AAGGA CATCC AGCAG					

FIG.7C

	730	740	750	760	770	780
XI12/8A	CAGAT GCGGG TGCCT GTCTG GGACA AGCCC ATGAG TCTGC CTGGG TACAT TGCGC GCCTT					
W22/1A	CAGAT GCGGG TGCCT GTCTG GGACA AGCCC ATGAG TCTGC CTGGG TACAT TGCGC GCCTT					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
B73/7-4	CAGAT GCGGG TGCCT GTCTG GGACA AGCCC ATGAG TCTGC CTGGG TACAT TGCGC GCCTT					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
XI12/8A	CAGAT GCGGG TGCCT GTCTG GGACA AGCCC ATGAG TCTGC CTGGG TACAT TGCGC GCCTT					
	790	800	810	820	830	840
XI12/8A	CCCAA GCCCC CTGGG ACTGA GTTGC TTGAG CAGGT GCTGC GTCTT GTTGG TGAAT CCCGG					
						>
X22/1A	CCCAA GCCCC CTGGG ACTGA GTTGC TTGAG CAGGT GCTGC GTCTT GTTGG TGAAT CCCGG					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
B73/7-4	CCCAA GCCCC CTGGG ACTGA GTTGC TTGAG CAGGT GCTGC GTCTT GTTGG TGAAT GCGGG					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
XI12/8A	CCCAA GCCCC CTGGG ACTGA GTTGC TTGAG CAGGT GCTGC GTCTT GTTGG TGAAT CCCGG					
	850	860	870	880	890	900
XI12/8A	CGCCC TGTTC TTTAT GTTGG CGGTG GCTGC GCAGC ATCTG GTGAG GAGTT GCGAC GCTTT					
						>
W22/1A	CGCCC TGTTC TTTAT GTTGG CGGTG GCTGC GCAGC ATCTG GTGAG GAGTT GCGAC GCTTT					
	11111 11111 11111 11 11 11111 11111 11111 11111 11111 11111 11111 11111					
B73/7-4	CGCCC TGTTC TTTAT GTGGG CGGTG GCTGC GCAGC ATCTG GTGAG GAGTT GCGAC GCTTT					
	11111 11111 11111 11 11 11111 11111 11111 11111 11111 11111 11111 11111					
XI12/8A	CGCCC TGTTC TTTAT GTTGG CGGTG GCTGC GCAGC ATCTG GTGAG GAGTT GCGAC GCTTT					
	910	920	930	940	950	960
XI12/8A	GTGGA GCTGA CTGGA ATCCC GGTCA CAACT ACTCT TATGG GCCTC GGCAA CTTCC CCAGC					
W22/1A	GTGGA GCTGA CTGGA ATCCC GGTCA CAACT ACTCT TATGG GCCTC GGCAA CTTCC CCAGC					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
B73/7-4	GTGGA GCTGA CTGGA ATCCC GGTCA CAACT ACTCT TATGG GCCTC GGCAA CTTCC CCAGC					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
XI12/8A	GTGGA GCTGA CTGGA ATCCC GGTCA CAACT ACTCT TATGG GCCTC GGCAA CTTCC CCAGC					

FIG.7D

	970	980	990	1000	1010	1020
XI12/8A	GACGA CCCAC TGTCT CTGCG CATGC TAGGT ATGCA TGGCA CGGTG TATGC AAATT ATGCA					
W22/1A	GACGA CCCAC TGTCT CTGCG CATGC TAGGT ATGCA TGGCA CGGTG TATGC AAATT ATGCA					
	11111 11111 11111 11111 11111 11111 11111 111 1 11111 11111 11111 11111					
B73/7-4	GACGA CCCAC TGTCT CTGCG CATGC TAGGT ATGCA TGGGA CGGTG TATGC AAATT ATGCA					
	11111 11111 11111 11111 11111 11111 11111 111 1 11111 11111 11111 11111					
XI12/8A	GACGA CCCAC TGTCT CTGCG CATGC TAGGT ATGCA TGGCA CGGTG TATGC AAATT ATGCA					
	1030	1040	1050	1060	1070	1080
XI12/8A	GTGGA TAAGG CCGAT CTGTT GCTTG CACTT GGTGT GCGGT TTGAT GATCG TGTGA CAGGG					
W22/1A	GTGGA TAAGG CCGAT CTGTT GCTTG CACTT GGTGT GCGGT TTGAT GATCG TGTGA CAGGG					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 1111 11111					
B73/7-4	GTGGA TAAGG CCGAT CTGTT GCTTG CACTT GGTGT GCGGT TTGAT GATCG CGTGA CAGGG					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 1111 11111					
XI12/8A	GTGGA TAAGG CCGAT CTGTT GCTTG CACTT GGTGT GCGGT TTGAT GATCG TGTGA CAGGG					
	1090	1100	1110	1120	1130	1140
XI12/8A	AAGAT TGAGG CTTT GCAAG CAGGG CTAAG ATTGT GCACG TTGAT ATTGA TCCGG CTGAG					
W22/1A	AAGAT TGAGG CTTT GCAAG CAGGG CTAAG ATTGT GCACG TTGAT ATTGA TCCGG CTGAG					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
B73/7-4	AAGAT TGAGG CTTT GCAAG CAGGG CTAAG ATTGT GCACG TTGAT ATTGA TCCGG CTGAG					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
XI12/8A	AAGAT TGAGG CTTT GCAAG CAGGG CTAAG ATTGT GCACG TTGAT ATTGA TCCGG CTGAG					
	1150	1160	1170	1180	1190	1200
XI12/8A	ATTGG CAAGA ACAAG CAGCC ACATG TGTCC ATCTG TGCAG ATGTT AAGCT TGCTT TGCAG					
W22/1A	ATTGG CAAGA ACAAG CAGCC ACATG TGTCC ATCTG TGCAG ATGTT AAGCT TGCTT TGCAG					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
B73/7-4	ATTGG CAAGA ACAAG CAGCC ACATG TGTCC ATCTG TGCAG ATGTT AAGCT TGCTT TGCAG					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
XI12/8A	ATTGG CAAGA ACAAG CAGCC ACATG TGTCC ATCTG TGCAG ATGTT AAGCT TGCTT TGCAG					

FIG.7E

	1210	1220	1230	1240	1250	1260						
XI12/8A	GGCAT	GAATG	CTCTT	CTTGA	AGGAA	GCACA	TCAAA	GAAGA	GCTTT	GACTT	TGGCT	CATGG
V22/1A	GGCAT	GAATG	CTCTT	CTTGA	AGGAA	GCACA	TCAAA	GAAGA	GCTTT	GACTT	TGGCT	CATGG
B73/7-4	GGCAT	GAATG	CTCTT	CTTGA	AGGAA	GCACA	TCAAA	GAAGA	GCTTT	GACTT	TGGCT	CATGG
XI12/8A	GGCAT	GAATG	CTCTT	CTTGA	AGGAA	GCACA	TCAAA	GAAGA	GCTTT	GACTT	TGGCT	CATGG

	1270	1280	1290	1300	1310	1320						
XI12/8A	AACGA	TGAGT	TGGAT	CAGCA	GAAGA	GGGAA	TTCCC	CCTTG	GGTAT	AAAAC	ATCTA	ATGAG
V22/1A	AACGA	TGAGT	TGGAT	CAGCA	GAAGA	GGGAA	TTCCC	CCTTG	GGTAT	AAAAC	ATCTA	ATGAG
B73/65	AACGA	TGAGT	TGGAT	CAGCA	GAAGA	GGGAA	TTCCC	CCTTG	GGTAT	AAAAC	ATCTA	ATGAG
XI12/8A	AACGA	TGAGT	TGGAT	CAGCA	GAAGA	GGGAA	TTCCC	CCTTG	GGTAT	AAAAC	ATCTA	ATGAG

	1330	1340	1350	1360	1370	1380						
XI12/8A	GAGAT	CCAGC	CACAA	TATGC	TATTC	AGGTT	CTTGA	TGAGC	TGACG	AAAGG	CGAGG	CCATC
V22/1A	GAGAT	CCAGC	CACAA	TATGC	TATTC	AGGTT	CTTGA	TGAGC	TGACG	AAAGG	CGAGG	CCATC
B73/7-4	GAGAT	CCAGC	CACAA	TATGC	TATTC	AGGTT	CTTGA	TGAGC	TGACG	AAAGG	CGAGG	CCATC
XI12/8A	GAGAT	CCAGC	CACAA	TATGC	TATTC	AGGTT	CTTGA	TGAGC	TGACG	AAAGG	CGAGG	CCATC

	1390	1400	1410	1420	1430	1440						
XI12/8A	ATCGG	CACAG	GTGTT	GGGCA	GCACC	AGATG	TGGGC	GGCAC	AGTAC	TACAC	TTACA	AGCGG
V22/1A	ATCGG	CACAG	GTGTT	GGGCA	GCACC	AGATG	TGGGC	GGCAC	AGTAC	TACAC	TTACA	AGCGG
B73/7-4	ATCGG	CACAG	GTGTT	GGGCA	GCACC	AGATG	TGGGC	GGCAC	AGTAC	TACAC	TTACA	AGCGG
XI12/8A	ATCGG	CACAG	GTGTT	GGGCA	GCACC	AGATG	TGGGC	GGCAC	AGTAC	TACAC	TTACA	AGCGG

FIG.7F

	1450	1460	1470	1480	1490	1500
X112/8A	CCAAG GCAGT GGTTC TCTTC AGCTG GTCTT GGGGC TATGG GATTT GGTTC GCCGG CTGCT					
W22/1A	CCAAG GCAGT GGTTC TCTTC AGCTG GTCTT GGGGC TATGG GATTT GGTTC GCCGG CTGCT					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
B73/7-4	CCAAG GCAGT GGTTC TCTTC AGCTG GTCTT GGGGC TATGG GATTT GGTTC GCCGG CTGCT					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
X112/8A	CCAAG GCAGT GGTTC TCTTC AGCTG GTCTT GGGGC TATGG GATTT GGTTC GCCGG CTGCT					
	1510	1520	1530	1540	1550	1560
X112/8A	GCTGG TGCTT CTGTG GCCAA CCCAG GTGTT ACTGT TGTTC ACATC GATGG AGATG GTAGC					
W22/1A	GCTGG TGCTT CTGTG GCCAA CCCAG GTGTT ACTGT TGTTC ACATC GATGG AGATG GTAGC					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
B73/7-4	GCTGG TGCTT CTGTG GCCAA CCCAG GTGTT ACTGT TGTTC ACATC GATGG AGATG GTAGC					
	11111 11111 11111 11 11 11111 1111 11111 11111 11111 11111 11111					
X112/8A	GCTGG TGCTT CTGTG GCCAA CCCAG GTGTT ACTGT TGTTC ACATC GATGG AGATG GTAGC					
	1570	1580	1590	1600	1610	1620
X112/8A	TTTCT CATGA ACGTT CAGGA GCTAG CTATG ATCCG AATTG AGAAC CTCCC GGTGA AGGTC					
W22/1A	TTTCT CATGA ACGTT CAGGA GCTAG CTATG ATCCG AATTG AGAAC CTCCC GGTGA AGGTC					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 1111 11111					
B73/7-4	TTTCT CATGA ACGTT CAGGA GCTAG CTATG ATCCG AATTG AGAAC CTCCC GGTGA AGGTC					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 1111 11111					
X112/8A	TTTCT CATGA ACGTT CAGGA GCTAG CTATG ATCCG AATTG AGAAC CTCCC GGTGA AGGTC					
	1630	1640	1650	1660	1670	1680
X112/8A	TTTGT GCTAA ACAAC CAGCA CCTGG GGATG GTGGT GCAGT GGGAG GACAG GTTCT ATAAG					
W22/1A	TTTGT GCTAA ACAAC CAGCA CCTGG GGATG GTGGT GCAGT GGGAG GACAG GTTCT ATAAG					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
B73/7-4	TTTGT GCTAA ACAAC CAGCA CCTGG GGATG GTGGT GCAGT GGGAG GACAG GTTCT ATAAG					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
X112/8A	TTTGT GCTAA ACAAC CAGCA CCTGG GGATG GTGGT GCAGT GGGAG GACAG GTTCT ATAAG					

FIG.7G

	1690	1700	1710	1720	1730	1740
XI12/8A	GCCAA CAGAG CGCAC	ACATA CTTGG	GAAAC CCAGA	GAATG AAAGT	GAGAT ATATC	CAGAT
W22/1A	GCCAA CAGAG CGCAC	ACATA CTTGG	GAAAC CCAGA	GAATG AAAGT	GAGAT ATATC	CAGAT
	11111 11111 11111	11111 11111 11111	11111 11111 11111	11111 11111 11111	11111 11111 11111	11111 11111 11111
B73/7-4	GCCAA CAGAG CGCAC	ACATA CTTGG	GAAAC CCAGA	GAATG AAAGT	GAGAT ATATC	CAGAT
	11111 11111 11111	11111 11111 11111	11111 11111 11111	11111 11111 11111	11111 11111 11111	11111 11111 11111
XI12/8A	GCCAA CAGAG CGCAC	ACATA CTTGG	GAAAC CCAGA	GAATG AAAGT	GAGAT ATATC	CAGAT

	1750	1760	1770	1780	1790	1800
XI12/8A	TTCGT GACGA TCGCC	AAAGG GTTCA	ACATT CCAGC	GGTCC GTGTG	ACAAA GAAGA	ACGAA
W22/1A	TTCGT GACGA TCGCC	AAAGG GTTCA	ACATT CCAGC	GGTCC GTGTG	ACAAA GAAGA	ACGAA
	11111 11111 11111	11111 11111 11111	11111 11111 11111	11111 11111 11111	11111 11111 11111	11111 11111 11111
B73/7-4	TTCGT GACGA TCGCC	AAAGG GTTCA	ACATT CCAGC	GGTCC GTGTG	ACAAA GAAGA	ACGAA
	11111 11111 11111	11111 11111 11111	11111 11111 11111	11111 11111 11111	11111 11111 11111	11111 11111 11111
XI12/8A	TTCGT GACGA TCGCC	AAAGG GTTCA	ACATT CCAGC	GGTCC GTGTG	ACAAA GAAGA	ACGAA

	1810	1820	1830	1840	1850	1860
XI12/8A	GTCCG CGCAG CGATA	AAGAA GATGC	TCGAG ACTCC	AGGGC CGTAC	CTCTT GGATA	TAATC
W22/1A	GTCCG CGCAG CGATA	AAGAA GATGC	TCGAG ACTCC	AGGGC CGTAC	CTCTT GGATA	TAATC
	11111 11111 11111	11111 11111 11111	11111 11111 11111	11111 11111 11111	11111 11111 11111	11111 11111 11111
B73/7-4	GTCCG CGCAG CGATA	AAGAA GATGC	TCGAG ACTCC	AGGGC CGTAC	CTCTT GGATA	TAATC
	11111 11111 11111	11111 11111 11111	11111 11111 11111	11111 11111 11111	11111 11111 11111	11111 11111 11111
XI12/8A	GTCCG CGCAG CGATA	AAGAA GATGC	TCGAG ACTCC	AGGGC CGTAC	CTCTT GGATA	TAATC

	1870	1880	1890	1900	1910	1920
XI12/8A	GTCCC ACACC AGGAG	CATGT GTTGC	CTATG ATCCC	TAATG GTGGG	GCTTT CAAGG	ATATG
				*		
W22/1A	GTCCC ACACC AGGAG	CATGT GTTGC	CTATG ATCCC	TA _g TG GTGGG	GCTTT CAAGG	ATATG
	11111 11111 11111	11111 11111 11111	11111 11111 11111	11111 11111 11111	11111 11111 11111	11111 11111 11111
B73/7-4	GTCCC ACACC AGGAG	CATGT GTTGC	CTATG ATCCC	TA _g TG GTGGG	GCTTT CAAGG	ATATG
	11111 11111 11111	11111 11111 11111	11111 11111 11111	11 11 11111	11111 11111 11111	11111 11111 11111
XI12/8A	GTCCC ACACC AGGAG	CATGT GTTGC	CTATG ATCCC	TAATG GTGGG	GCTTT CAAGG	ATATG

FIG.7H

	1930	1940	1950	1960						
XI12/8A	ATCCT	GGATG	GTGAT	GGCAG	GA CTG	TGTAC	TGATC	TAAAA	TCCAG	CAAG
W22/1A	ATCCT	GGATG	GTGAT	GGCAG	GA CTG	TGTAC	TGATC	TAAAA	TCCAG	CAAG>
	11111	11111	11111	11111	11111	11111	11111	11111	11111	1111
B73/7-4	ATCCT	GGATG	GTGAT	GGCAG	GA CTG	TGTAC	TGATC	TAAAA	TCCAG	CAAG>
	11111	11111	11111	11111	11111	11111	11111	11111	11111	1111
XI12/8A	ATCCT	GGATG	GTGAT	GGCAG	GA CTG	TGTAC	TGATC	TAAAA	TCCAG	CAAG

FIG.7 I

	10	20	30	40	50	60
XI12/8A	MATAA AASTA LTGAT TAAPK ARRRRA HLLAT RRALA APIRC SAASP AMPMA PPATP LRPVG					
V22/1A	MATAA AASTA LTGAT TAAPK ARRRRA HLLAT RRALA APIRC SAASP AMPMA PPATP LRPVG 11111 11111 111 1 11111 11111 11111 11111 11111 11111 11111 11111					
B73/7-4	MATAA AASTA LTGAT TAAPK ARRRRA HLLAT RRALA APIRC SAASP AMPMA PPATP LRPVG 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
XI12/8A	MATAA AASTA LTGAT TAAPK ARRRRA HLLAT RRALA APIRC SAASP AMPMA PPATP LRPVG					
	70	80	90	100	110	120
XI12/8A	PTDPR KGADI LVESL ERCGV RDVFA YPGGA SMEIH QALTR SPVIA NHLFR HEQGE AFAAS #					
V22/1A	PTDPR KGADI LVESL ERCGV RDVFA YPGGA SMEIH QALTR SPVIA NHLFR HEQGE AFAAS 11 11 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
B73/7-4	PTePR KGADI LVESL ERCGV RDVFA YPGGA SMEIH QALTR SPVIA NHLFR HEQGE AFAAS 11 11 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
XI12/8A	PTDPR KGADI LVESL ERCGV RDVFA YPGGA SMEIH QALTR SPVIA NHLFR HEQGE AFAAS					
	130	140	150	160	170	180
XI12/8A	GYARS SGRVG VCIAT SGPGA TNLVS ALADA LLDSV PMVAI TGOVP RRMIG TDAFQ ETPIV					
V22/1A	GYARS SGRVG VCIAT SGPGA TNLVS ALADA LLDSV PMVAI TGOVP RRMIG TDAFQ ETPIV 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
B73/7-4	GYARS SGRVG VCIAT SGPGA TNLVS ALADA LLDSV PMVAI TGOVP RRMIG TDAFQ ETPIV 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
XI12/8A	GYARS SGRVG VCIAT SGPGA TNLVS ALADA LLDSV PMVAI TGOVP RRMIG TDAFQ ETPIV					
	190	200	210	220	230	240
XI12/8A	EVTRS ITKHN YLVLD VDDIP RVVQE AFFLA SSGRP GPVLV DIPKD IQQQM AVPVW DKPMS					
V22/1A	EVTRS ITKHN YLVLD VDDIP RVVQE AFFLA SSGRP GPVLV DIPKD IQQQM AVPVW DKPMS 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
B73/7-4	EVTRS ITKHN YLVLD VDDIP RVVQE AFFLA SSGRP GPVLV DIPKD IQQQM AVPVW DKPMS 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
XI12/8A	EVTRS ITKHN YLVLD VDDIP RVVQE AFFLA SSGRP GPVLV DIPKD IQQQM AVPVW DKPMS					

FIG.8A

	250	260	270	280	290	300						
XI12/8A	LPGYI	ARLPK	PPATE	LLEQV	LRLVG	ESRRP	VLYVG	GGCAA	SGEEL	RRFVE	LTGIP	VTTTL
V22/1A	LPGYI	ARLPK	PPATE	LLEQV	LRLVG	ESRRP	VLYVG	GGCAA	SGEEL	RRFVE	LTGIP	VTTTL
B73/7-4	LPGYI	ARLPK	PPATE	LLEQV	LRLVG	ESRRP	VLYVG	GGCAA	SGEEL	RRFVE	LTGIP	VTTTL
XI12/8A	LPGYI	ARLPK	PPATE	LLEQV	LRLVG	ESRRP	VLYVG	GGCAA	SGEEL	RRFVE	LTGIP	VTTTL

	310	320	330	340	350	360						
XI12/8A	MGLGN	FPSDD	PLSLR	MLGMH	GTVYA	NYAVD	KADLL	LALGV	RFDDR	VTGKI	EAFAQ	RAKIV
V22/1A	MGLGN	FPSDD	PLSLR	MLGMH	GTVYA	NYAVD	KADLL	LALGV	RFDDR	VTGKI	EAFAQ	RAKIV
B73/7-4	MGLGN	FPSDD	PLSLR	MLGMH	GTVYA	NYAVD	KADLL	LALGV	RFDDR	VTGKI	EAFAQ	RAKIV
XI12/8A	MGLGN	FPSDD	PLSLR	MLGMH	GTVYA	NYAVD	KADLL	LALGV	RFDDR	VTGKI	EAFAQ	RAKIV

	370	380	390	400	410	420						
XI12/8A	HVDID	PAEIG	KNKQP	HVSIC	ADVKL	ALQGM	NALLE	GSTSK	KSDFD	GSWND	ELDQO	KREFP
V22/1A	HVDID	PAEIG	KNKQP	HVSIC	ADVKL	ALQGM	NALLE	GSTSK	KSDFD	GSWND	ELDQO	KREFP
B73/7-4	HVDID	PAEIG	KNKQP	HVSIC	ADVKL	ALQGM	NALLE	GSTSK	KSDFD	GSWND	ELDQO	KREFP
XI12/8A	HVDID	PAEIG	KNKQP	HVSIC	ADVKL	ALQGM	NALLE	GSTSK	KSDFD	GSWND	ELDQO	KREFP

	430	440	450	460	470	480						
XI12/8A	LGYKT	SNEEI	QPQYA	IQVLD	ELTKG	EATIG	TGVGO	HOMWA	ADYYT	YKRPR	QVLSS	AGLGA
V22/1A	LGYKT	SNEEI	QPQYA	IQVLD	ELTKG	EATIG	TGVGO	HOMWA	ADYYT	YKRPR	QVLSS	AGLGA
B73/7-4	LGYKT	SNEEI	QPQYA	IQVLD	ELTKG	EATIG	TGVGO	HOMWA	ADYYT	YKRPR	QVLSS	AGLGA
XI12/8A	LGYKT	SNEEI	QPQYA	IQVLD	ELTKG	EATIG	TGVGO	HOMWA	ADYYT	YKRPR	QVLSS	AGLGA

FIG.8B

	490	500	510	520	530	540
XI12/8A	MGFGL PAAAG ASYAN PGVTV VDIDG DGSFL MNVQE LAMIR IENLP VKVFE LNNQH LGMVV					
W22/1A	MGFGL PAAAG ASYAN PGVTV VDIDG DGSFL MNVQE LAMIR IENLP VKVFE LNNQH LGMVV					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
B73/7-4	MGFGL PAAAG ASYAN PGVTV VDIDG DGSFL MNVQE LAMIR IENLP VKVFE LNNQH LGMVV					
	11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
XI12/8A	MGFGL PAAAG ASYAN PGVTV VDIDG DGSFL MNVQE LAMIR IENLP VKVFE LNNQH LGMVV					
	550	560	570	580	590	600
XI12/8A	QVEDR FYKAN RAHTY LGNPE NESEI YPDFV TIAKG FNIPA VRYTK KNEVR AAIKK MLETP					
W22/1A	QVEDR FYKAN RAHTY LGNPE NESEI YPDFV TIAKG FNIPA VRYTK KNEVR AAIKK MLETP					
	1 111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
B73/7-4	QVEDR FYKAN RAHTY LGNPE NESEI YPDFV TIAKG FNIPA VRYTK KNEVR AAIKK MLETP					
	1 111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111 11111					
XI12/8A	QVEDR FYKAN RAHTY LGNPE NESEI YPDFV TIAKG FNIPA VRYTK KNEVR AAIKK MLETP					
	610	620	630			
XI12/8A	GPYLL DIIVP HQEHV LPMIP NGGAF KDMIL DGDGR TVY*					
W22/1A	GPYLL DIIVP HQEHV LPMIP sGGAF KDMIL DGDGR TVY>					
	11111 11111 11111 11111 11111 11111 11111 111					
B73/7-4	GPYLL DIIVP HQEHV LPMIP sGGAF KDMIL DGDGR TVY>					
	11111 11111 11111 11111 1111 11111 11111 111					
XI12/8A	GPYLL DIIVP HQEHV LPMIP NGGAF KDMIL DGDGR TVY					

FIG.8C

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(54) **Imidazolinone resistant ahas mutants.**

(57) The present invention relates to monocot genes encoding a mutant AHAS enzyme that is specifically resistant to imidazolinone herbicides. Exemplary of these genes are corn DNA sequences which encode an amino acid substitution at position 621 of the wild-type AHAS enzyme. The mutant gene can be used to transform other plants to herbicide resistance; in this regard, the invention also provides host cells and vectors containing the gene, which cells and vectors are useful in the transformation process.

EP 0 525 384 A3



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CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons A : member of the same patent family, corresponding document			

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